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LYMPHOEPITHELIOMA *

JAMES EWING, M.D.

(From the Pathological Laboratory of the Memorial Hospital, New York, N. Y.)

Lymphoepithelioma is a term employed by Regaud [1] and by Schmincke [2] in 1921 to designate certain tumors of mucous surfaces of the nasopharynx, which exhibit a specific structure and run a somewhat peculiar clinical course. Radiosensitivity was the main feature which led the observers to recognize in the tumors a peculiar clinical and pathological type.

The tumors occur at all ages, but are especially frequent between 30 and 60 years. They produce rather small local growths which are soft, slow to ulcerate, do not bleed much, and are therefore often overlooked and nearly always difficult to detect. The first symptom is often an enlargement of the cervical lymph nodes, multiple or bilateral, which may attain considerable dimensions before the primary tumor is detected. Distant metastases occur in the bones and in the liver, so that the course is usually fatal, although prolonged. The duration is generally about three years. The marked subsidence of the tumors under external radiation led the observers to conclude that the tumors were essentially different from the ordinary epidermoid carcinoma.

Support for this belief was obtained from the work of Jolly [3] and Mollier,[4] who have maintained that certain lymphoid structures in mucous membranes are covered with stratified epithelium which has a peculiar physiological relation to lymphocytes. Over Peyer's patches and the faucial and lingual tonsils, the epithelial layer is altered by the normal infiltration of lymphocytes. In the thymus the entire reticulum is of epithelial origin. The epithelial layer is

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generally thickened at the points where the lymphoid infiltration occurs. Intermediate grades of epithelial plication and lymphoid infiltration are observed in the bursa of Fabricius of birds, and in the pharyngeal tonsils. Hence Jolly designated these organs as lymphoepithelial.

In the human tumors derived from these organs the epithelial cells fail to assume squamous characters but remain undifferentiated, of rather large size, often forming syncytia, and are always accompanied by growth of lymphocytes. They may thus be difficult to distinguish from lymphosarcoma, but as a rule the epithelial elements are easily recognized as sheets of large flat cells.

The conception of lymphoepithelial organs, however, appears to be much older than Jolly's studies, 1914. Between 1886 and 1906 an active debate was carried on by Stöhr,[5] and others, and by Retterer,[6] the former holding that the lining epithelium of the tonsils is confined to the surface of the organ, while Retterer maintained that much of the reticulum of the tonsils is of epithelial nature and similar to the reticulum of the thymus. Jurisch[7] reviewed this subject at length. Retterer believed that in the tonsils and other superficial lymphoid structures there was the same intimate relation between epithelial reticulum and lymphocytes as in the thymus. At present, the evidence is against the participation of epithelium in the reticular structure of any lymphoid organ except the thymus, but it remains somewhat obscure why the thymus is the only lymphoid organ to enjoy this peculiar relation. It is also of interest to note that some tumors of the thymus present the most characteristic features of lymphoepithelioma.

The peculiar structural characters of certain epidermoid carcinomas of the nasopharynx have been noted by several observers, including Crowe and Baylor,[8] and New,[9] who reported several cases of this type and mentioned their resemblance to lymphosarcoma and the difficulty of finding the primary tumor. At the Memorial Hospital a group of radiosensitive nasopharyngeal tumors has long been recognized as peculiar in structure and as highly radiosensitive, and a series of such cases was reported by Quick and Cutler[10] under the term "transitional cell epidermoid carcinoma." This term was used because the grounds for assuming an origin from specialized epithelium as described by Regaud were regarded as not yet fully established. Their tumors arose from the tonsil, base of tongue and

nasopharynx, where stratified epithelium of transitional cell type is present.

A full exposition of lymphoepithelioma is furnished by Jovin,[11] who reports eight cases and discusses the theory of origin, the structure, and the clinical features. In his cases the tumor cells grew in wide sheets which were infiltrated with many lymphocytes (Figs. 3, 4, 5). The looser structure (Fig. 6), described by Schmincke and other observers, was not encountered. Six of the cases died from recurrences and usually with extensive metastases to the bones, but there were no autopsies. Two cases recovered after external radiation.

In Schmincke's cases the masses of epithelial cells were very loose and broken up by very rich admixture of lymphocytes, so that the structure resembled lymphosarcoma. Schmincke considered the question whether the tumors were not of mixed origin involving both epithelium and lymphocytes.

Derigs [12] reports a typical case arising in the pharyngeal wall of a subject of 15 years, with extensive metastases to bones, lungs and liver. The structure was very loose, the syncytial masses being broken up by many lymphocytes. She considered that the tumor process resided in the epithelium which, in all the metastases exerted a chemotactic attraction for lymphocytes.

Discussion

In a study of epithelial tumors of the tonsils and nasopharynx, the writer has been confronted with several questions which have considerable practical importance and the elucidation of which may have a bearing on the question of lymphoepithelioma.

1. Radiosensitivity is not an exclusive feature of lymphoepithelioma or of any one type of epidermoid carcinoma of the nasopharynx. Highly anaplastic carcinomas of tongue, tonsil and nares, which grow rapidly and metastasize widely, may melt down under moderate radiation. Very malignant tumors of this nature are also encountered in the uterus, esophagus, and pharynx. They produce relatively small primary growths, the metastases are very early and widespread, the cells are small, often rounded, resembling lymphosarcoma, and the response to radiation is very notable. In the group of cervical uterine growths, Cutler found a high proportion of three-year cures by radiation. It is possible that these tumors may be

distinguished from lymphoepithelioma by their more rapid course and by the absence of lymphocytes in the tumor tissue, but not by a difference in radiosensitivity.

2. Radiosensitivity may depend on embryonal qualities of the cells of origin, and the question arises whether any of the tumors of the nasopharynx arise from embryonal cells. The evidence in favor of this view is not very definite, but there is a well known group of tumors which may be called adenoid cystic epithelioma, which Krompecher classed with the basal cell group and which arise from the mucous glands of the nasopharynx. These may possibly be regarded as of embryonal type. They grow slowly and usually produce bulky tumors, but the metastases are late. They are moderately radiosensitive. The cells are small and appear in sheets inclosing mucous globules or larger cysts containing mucus. A resemblance to thyroid tissue is sometimes mentioned. They frequently recur after operation so that the eventual prognosis is serious. We have observed two such cases which recurred persistently after operation and radiation and eventually proved fatal, eleven and thirteen years after onset, and with very numerous metastases throughout the lungs and bone marrow. I think the metastases resulted from the extensive development of dilated veins about the tumor nodules into which the tumor cells forced a way. There is little difficulty in separating these growths from other types of epidermoid carcinoma and especially from lymphoepithelioma.

3. In the study of nasal carcinoma we have gradually reached the conclusion that the majority of them arise from the ordinary transitional epithelium which lines the Schneiderian membrane and the pharyngeal crypts and sinuses. This view appears to have been adopted by many observers, and the characters of Schneiderian carcinoma are well known. They are very cellular, growing at times diffusely, almost like lymphosarcoma, and the epithelial qualities of the cells are not prominent, or are entirely absent. The growth does not appear to be accompanied by infiltration of lymphocytes. There are doubtless many grades of malignancy with corresponding variations in the rate of growth but most of the tumors are recognized early because of ulceration and bleeding, and they progress steadily and often rapidly. It has often been noted that lymphatic metastases in this group are generally late.

4. The ducts of mucous glands at the base of the tongue and

throughout the pharyngeal wall are a possible source of transitional cell carcinoma, and there is considerable evidence that some tumors arise from this source. At the base of the tongue they may grow for some months before reaching the surface. Lymphatic metastases may therefore occur before the primary tumor is recognized, and in this respect they resemble lymphoepithelioma. In the metastases, epidermoid characters are usually distinct, but the growth may be very diffuse and it has proved impossible in our hands to distinguish some of these growths from the descriptions of lymphoepithelioma. The participation of lymphocytes in the primary or secondary tumors has not been observed; but bulky metastases in the liver or lungs have been a notable feature.

The radiosensitivity of Schneiderian and transitional cell carcinoma is very marked. The local growths may often be controlled by external radiation, but the outlook is unfavorable because of the metastases.

5. Carcinoma of the nasopharynx frequently occurs in young subjects between the ages of 10 and 20 years. The structure of these tumors resembles that of transitional cell carcinoma or lymphoepithelioma. The tumors grow slowly, but they eventually reveal their presence by nasal obstruction and bleeding. In many cases, however, the first sign of the disease is the swelling of cervical lymph nodes, but on account of the early age of the patients tuberculosis is usually suspected. In one case bulky metastases occurred in the liver, this organ being almostly completely replaced by tumor tissue (Figs. 7, 8).

The tumor cells are generally small, the nuclei relatively large and very hyperchromatic, and the cells form sheets or cords with comparatively little stroma reaction. I have seldom observed any definite admixture of lymphocytes. The distant metastases may be explained by the rich development of blood channels, some of which may be lined by tumor cells in immediate contact with the circulating blood.

The radiosensitivity is high, and after heavy external treatment considerable sloughing may occur. The outcome of many cases is unfavorable. I have been unable to trace the exact origin of the growths, but find that they occur at almost any point in the nasal passages or upper pharyngeal wall. It seems impossible to separate them from lymphoepithelioma or Schneiderian carcinoma.

6. In certain nasopharyngeal carcinomas one finds quite different structures in different parts of the same tumor, so that its classification becomes uncertain. I have observed two distinct structural types in the same carcinoma occurring in the nasopharynx of a young subject. In one area there were very small cells lining a blood sinus, while another area was composed of large flat cells approaching the squamous type. The occurrence of such cases suggests that the particular cell form of a tumor may not give a definite indication of its exact origin.

In a case of carcinoma of the frontal sinus there were areas of adenoma malignum, foci of adult cells approaching the squamous type, and small strands of small cells resembling transitional epithelium.

In the metastases the structure may be more anaplastic and undifferentiated, or less so. In a tumor of the base of the tongue showing a structure of transitional cell carcinoma or lymphoepithelioma, the structure in the cervical nodes was much more adult in type approaching squamous carcinoma in places, while bulky metastases in the liver were extremely cellular and undifferentiated. Since the metastases in lymph nodes are free from secondary changes due to infection and ulceration, the structure in the nodes should reflect more truly the original cell type.

7. The frequent occurrence of precocious metastases in cervical lymph nodes when a primary tumor of the nasopharynx exists, but long escapes detection, makes a heavy inroad into the field of so-called primary endothelioma of lymph nodes and renders the diagnosis of endothelioma of cervical lymph nodes a hazardous step. It is clear that a diagnosis of such a tumor in the neck cannot be accepted unless a long period elapses to permit the primary tumor to appear, or unless an autopsy proves the absence of such primary carcinoma. Moreover, the structure of certain transitional cell carcinomas or lymphoepitheliomas so closely resembles that which many authors including the writer have been in the habit of regarding as endothelioma, that the separation of the two groups of tumors on histological features must be regarded as extremely difficult, or often impossible. The mode of invasion of lymphoid tissue by certain lymphoepitheliomas is very peculiar, since it produces a structure which seems to spring directly from the lymphoid reticulum. There seems to be no way to determine whether these large

cells uniformly diffused through a lymphoid tissue are invading epithelium or multiplying reticulum cells. The chances favor a metastatic tumor in all such questionable cases.

Nevertheless, I continue to encounter occasional tumors of lymph nodes of the neck and other regions in which no primary tumor is discovered during a long period of the life of the patient or at autopsy, and which it seems necessary to regard as instances of primary endothelioma. The gross anatomy of the cases of endothelioma described by von Recklinghausen,[18] and others, especially those occurring in the abdominal region, is quite different from the conditions seen in metastatic carcinoma. There seems to be little doubt that primary endothelioma of lymph nodes does occur, but the scope of this group of tumors needs reconsideration in the light of the present knowledge of lymphoepithelioma.

The separation of cellular carcinoma of the tonsil from lymphosarcoma is also often difficult. Yet, in the epithelial tumors certain areas nearly always show sheets of cells of definite polyhedral form, while in lymphosarcoma the cells may be large but they never occur in sheets or with definite epithelioid characters. The gross anatomy and the clinical course of the two diseases are generally quite different.

Tumors of the tonsils and nasopharynx which may with reasonable accuracy be classed as lymphoepithelioma are comparatively rare, while tumors of transitional epithelium in which lymphocytes play no part are relatively common. As evidence on this point, I have reviewed 200 cases of epithelioma of the tonsils and base of tongue, and 100 cases of nasopharyngeal carcinoma, from the records of the Memorial Hospital, with the following result:

Tumors of tonsils and base of tongue	200 cases
Squamous epithelioma	72 per cent
Transitional cell carcinoma	12 " "
Lymphosarcoma	9 " "
Lymphoepithelioma	4 " "
Unclassified	3 " "
Nasopharyngeal tumors	100 cases
Squamous carcinoma	30 per cent
Transitional cell	37 " "
Lymphosarcoma	15 " "
Lymphoepithelioma	11 " "
Adenoma malignum	3 " "
Adenoid cystic epithelioma	4 " "

During this study it became apparent that there must be considerable personal equation entering into the decision as to which category certain tumors belong. Considerable difficulty was experienced in deciding on histological grounds whether the tumor was a lymphosarcoma or a carcinoma. In many cases clinical evidence seemed to be necessary to make this decision. The group classified as lymphoepithelioma did not appear to be very sharply defined, and it was at times difficult to decide whether to class the case with lymphoepithelioma, transitional cell carcinoma of Schneiderian origin, or as lymphosarcoma. The evidence furnished by several autopsies seemed inadequate to decide the question, since bulky metastases in the liver and bones occurred in all but lymphosarcomas.

I have had opportunity to study many sections of lymphoepithelioma with Dr. Laccasagne at the Radium Institute in Paris. I find that he includes under the term lymphoepithelioma, the various tumors which I have been designating as transitional cell carcinoma, and Schneiderian carcinoma.

Summary

The present available data seem to call for the recognition of a particular form of epidermoid carcinoma occurring especially in the nasopharynx and designated by Regaud and Schmincke as lymphoepithelioma. The tumors arise from modified epithelium overlying lymphoid structures in tonsils, base of tongue and nasopharynx. They occur at all ages, frequently in young subjects, grow slowly, are often overlooked, tend to produce early metastases in the neck, and later widespread extensions to liver and bone marrow, and are generally fatal. They exhibit a marked primary response to radiation. The diagnosis must be based on the structure, which shows sheets of pale staining epithelial cells, often in syncytia, and infiltrated with many lymphocytes, both in the primary tumor and in metastases. The diagnosis may not be based on clinical appearance, or course, or location, or radiosensitivity. Other tumors of the same regions are radiosensitive, produce early metastases to lymph nodes, bones, and viscera. Anaplastic carcinomas may be distinguished by their more rapid course and cellular structure.

The exact scope of lymphoepithelioma is not yet clear. Tumors of

transitional epithelium located in many areas of the nasopharynx and lining the ducts of mucous glands, seem to produce tumors showing many of the characteristics of lymphoepithelioma. It seems possible that the admixture of lymphocytes may be the result of a low grade inflammatory process occurring in certain other tumors, but their appearance in metastases is probably not to be explained in this way.

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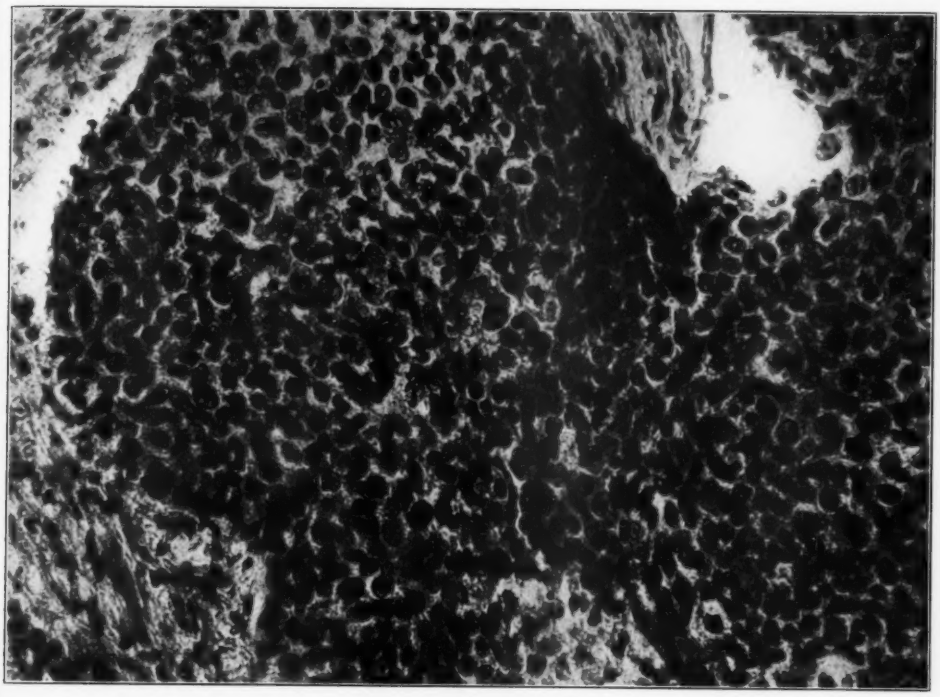
DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Epidermoid carcinoma of tonsil. Solid cords of coherent cells. Epithelial characters distinct. Transitional cell carcinoma.

FIG. 2. Epidermoid carcinoma of nasopharynx. Broad sheets of loosely coherent cells. Epithelial characters fairly distinct. Transitional cell carcinoma.



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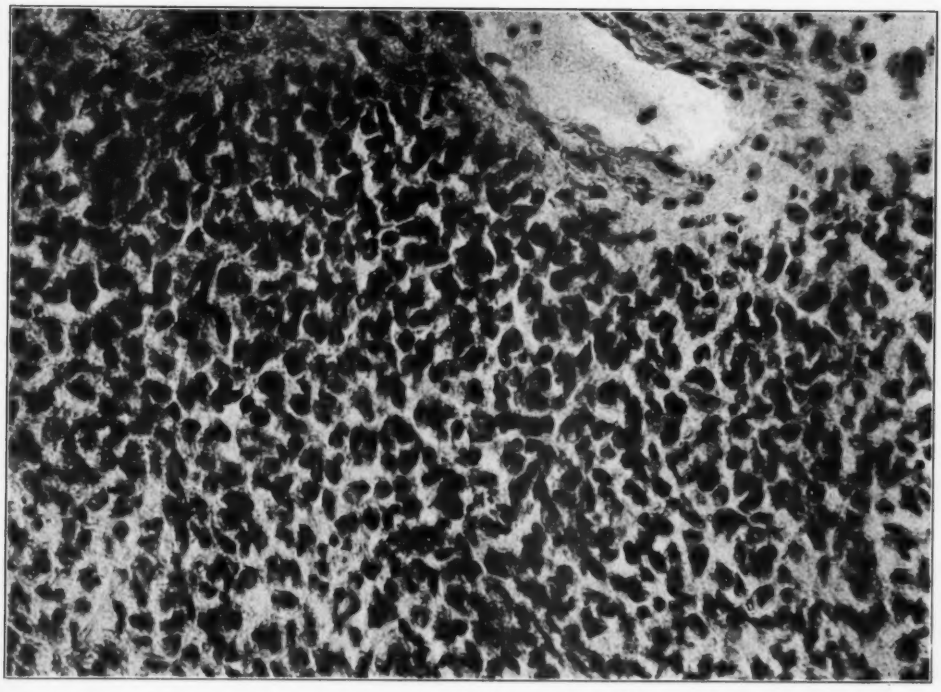


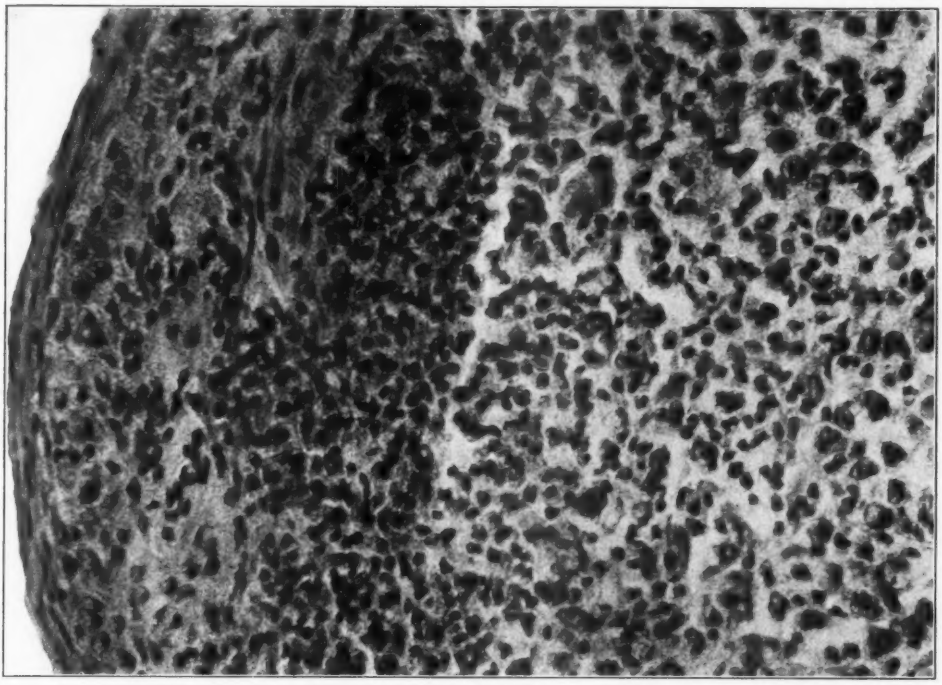
2

Ewing Lymphoepithelioma

PLATE 21

FIG 3. Epidermoid carcinoma. Indistinct alveolar grouping of small polyhedral cells. Slight infiltration of lymphocytes.

FIG. 4. Epidermoid carcinoma of nares. Lymphoepithelioma of Regaud. Syncytial masses of large polyhedral cells with large nuclei. Rich infiltration by lymphocytes. Patient well three years after radiation.



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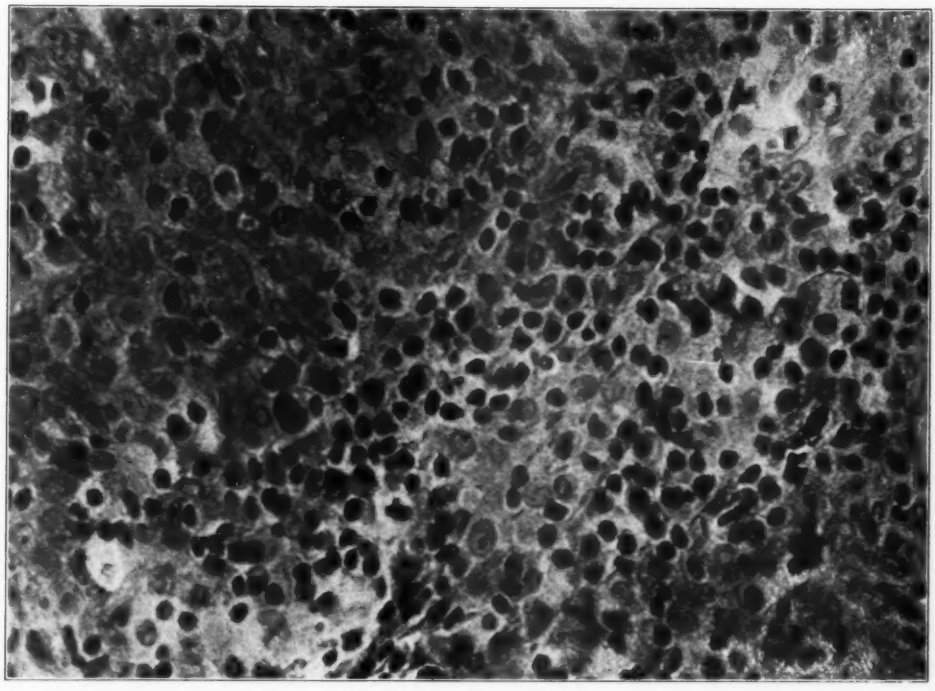

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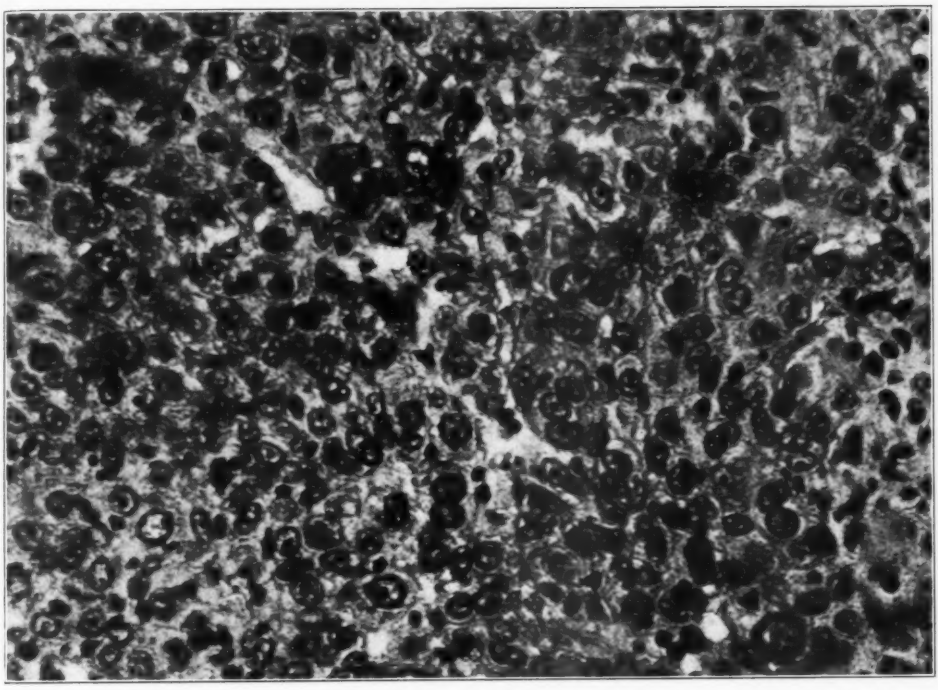
Ewing Lymphoepithelioma

PLATE 22

FIG. 5. Epidermoid carcinoma of pharynx. Lymphoepithelioma of Regaud. Syncytial masses of large polyhedral cells. Infiltration by lymphocytes.

FIG. 6. Epidermoid carcinoma of nasopharynx. Lymphoepithelioma of Schmincke. Broad areas of loose hyperchromatic cells of indistinct epithelial type. The structure somewhat resembles lymphosarcoma.



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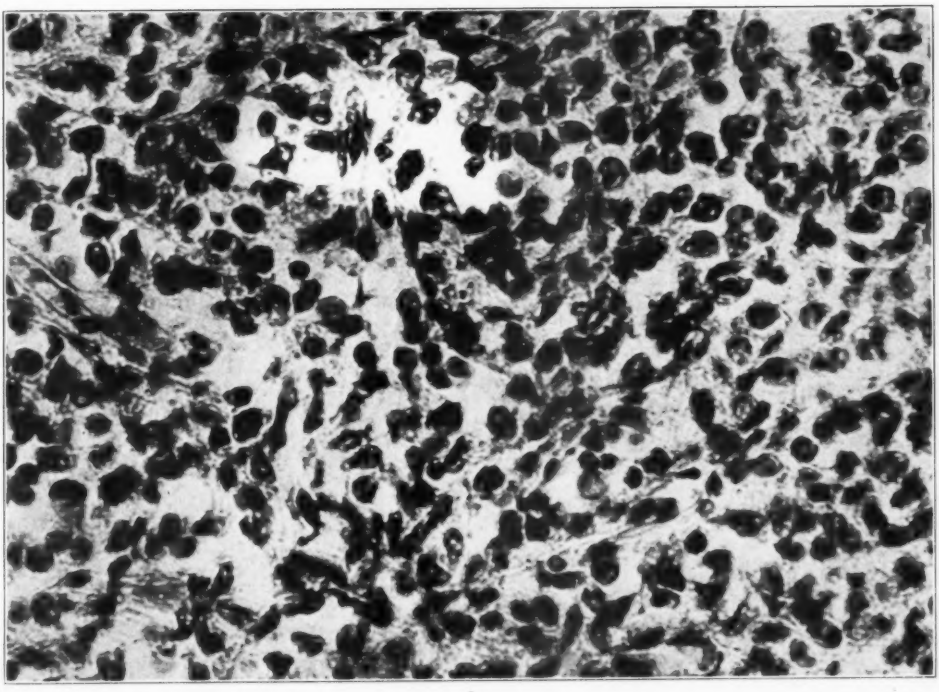

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Ewing

Lymphoepithelioma

PLATE 23

FIG. 7. Epidermoid carcinoma of nares in a subject 23 years of age. Lympho-epithelioma. Diffuse infiltration of entire liver.

FIG. 8. Epidermoid carcinoma of nares in a subject 23 years of age. Lympho-epithelioma. Structure of metastasis in liver.

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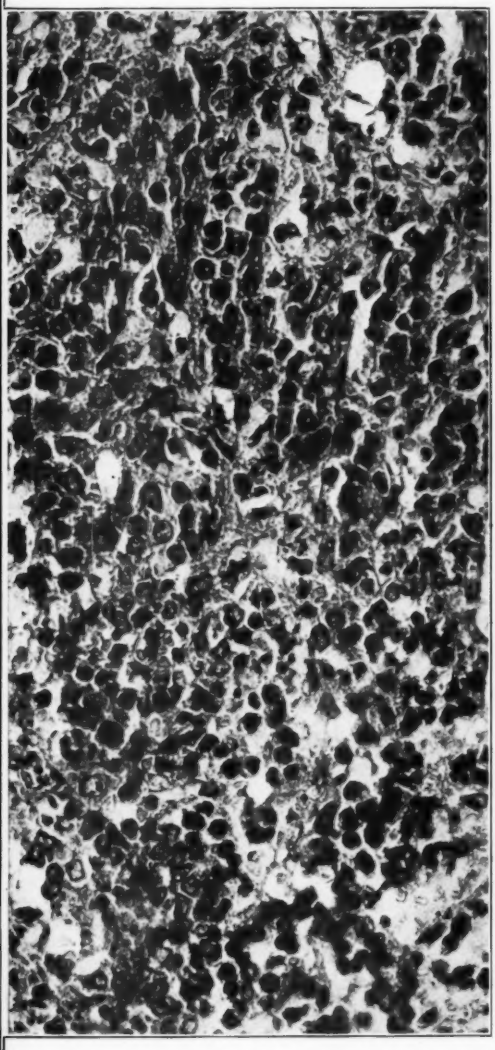
Ewing



8

Lymphoepithelioma

BIOPSY HISTOLOGY IN THE GRADING OF RECTAL CARCINOMA *

FRED W. STEWART, M.D., AND JOHN W. SPIES, M.D.

(From the Pathology Laboratory of the Memorial Hospital, and the Department of Pathology, Cornell University Medical College, New York City)

INTRODUCTION

In 1915 Broders began the practice of grading neoplasms on purely histological grounds and a series of studies [1, 2, 3, 4] by this author leaves very little question of the value of histologic grading of degree of malignancy in interpreting the probable course a given tumor type may be likely to pursue in a group of individuals. Like all purely laboratory methods it is apt to fail in the individual case wherein due and proper attention is not given to other influencing factors. A general review of the question of histologic prognosis of tumors is given by Plaut [5] and objections to the use of the method in individual cases in the absence of consideration of size and location of tumors, duration of disease and numerous other factors, are fully discussed. Apparently Plaut is not overimpressed with the value of the histologic prognosis alone in the individual patient.

Rankin and Broders [6] have recently applied Broders' method of tumor grading to a large series of rectal carcinomas. The material for grading came from 598 surgical excisions at the Mayo clinic. Upon the basis of histologic structure the tumors were grouped into four grades; the results would appear to be of distinct aid in estimating the probable operative result obtainable from the average patient.

We were interested in comparing the results obtained by Broders from a study of operative material, presumably affording opportunity for complete tumor examination and classification in accordance with the full pathological findings, with results obtained from examination of small, usually superficial, biopsy specimens. Since at the Memorial Hospital rectal carcinoma is but rarely excised, most of the cases falling within the inoperable group as judged by surgeons of the rectal clinic, almost no operative material is available for study. Furthermore the hospital has very little bed space avail-

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able for treatment of cancer in its terminal stages, and consequently but few autopsies have been obtainable. On the other hand some cases, while undergoing radiation therapy in the clinic, have had repeated biopsies thus affording a certain amount of material for the study of the question of change of tumor type during the course of the disease.

We have endeavored to determine as nearly as possible from our material the answers to the following questions: (1) the approximate number of tumors falling in each group; (2) the relation of age to tumor type; (3) the influence, if any, of site of tumor to histologic type; (4) the question of tumor type and duration of symptoms before the patient enters the clinic; (5) the duration of life from the onset of symptoms in cases of the various types dying of the disease; (6) metastases found at colostomy in each of the four groups; (7) change of tumor type as observed in repeated biopsies; (8) an attempted analysis of those cases in which marked discrepancy existed between the clinical course of the disease and the average clinical course which the disease should have run had it behaved in accordance with the tumor type.

Grading of Tumors

It is first desirable to explain, mainly photomicrographically, the grouping of the tumors. In general the grading we have employed approximates rather closely that of Broders and Rankin, but at the outset it must be stated that no very sharp lines can be drawn; what one sees is a series of gradual transformations in the direction of increasing anaplasia as the tumor grade changes from I to IV. There is room therefore for many individual variations in the grading and what we have sought are broad groups, necessarily with some overlapping in both directions, rather than hard and fast lines of division which cannot exist clinically.

Grade I: Under Grade I we have placed those tumors with malignant features, yet structurally approaching the benign adenoma. In tumors of this type the epithelial cells line distinct glandular cavities (Fig. 1). A papillary arrangement is frequently, almost uniformly present. Invasive tendencies, so far as determinable from the biopsy specimen, are negligible features. The epithelium contains two or three layers of nuclei, elongated, of rather uniform size,

without distinct hyperchromatism; mitoses are present but not numerous. The cell polarity is preserved, and the nuclei occupy the basal regions of the cells, leaving a cytoplasmic zone bordering the lumen of the gland. Secretory activity may or may not be present.

Grade II: In Grade II the tumor cells still line definite glandular cavities; these cavities are more irregular; there is a marked tendency toward the production of papillary ingrowths and outpocketings of varying sizes and shapes. The walls of the glandular cavities are thicker; nuclei are arranged in three or more layers; they are more hyperchromatic, larger, more elongated, and are situated through the entire thickness of the epithelium; no very distinct cytoplasmic border separates the nuclear layers from the gland lumina; evidences of ulceration and infection are fairly common; these tumors are classified as adenoma malignum or adenoma destruens, Grade II (Fig. 2). The beginning distribution of nuclei throughout the thickness of the gland wall may be interpreted as being an early expression of loss of cell polarity. Mitoses are often numerous.

Grade III: Tumors classified as Grade III (Figs. 3 and 4) show in addition to glandular cavities resembling those seen in Grade II, solid areas where glandular tendencies are either lost or are represented by abortive attempts at tubule formation; the cells may be small with hyperchromatic nuclei, or they may be quite large and spindle-shaped. In either case there is definite loss of cell polarity. Papillary characters are poorly marked or absent. The stroma is frequently markedly increased, whereas in the two preceding groups it is a less prominent feature. Often the strands of tumor cells present a marked elongated, drawn-out appearance suggestive of infiltrating tendencies. Ulceration and infection are frequent, and the feeling exists that when infection is marked the tumor grade for clinical purposes should be increased. Thus Figs. 5 and 6 typify tumors which ordinarily would have been given as Grade III but which were advanced in grade in view of the increased clinical malignancy resulting from infection. The clinical course of the two cases fully justified this advance in grading. Why infection should alter the clinical course in the direction of increased malignancy is not known; we do not know nor do we see any way of determining from our material whether the infection hastens the lethal termination by means of increased metastases or merely through the local and

constitutional effects of such infection, for example hemorrhage and toxemia.

Grade IV: Grade IV comprises a heterogeneous group of tumors; some of these suggest that with the accumulation of more material a fifth grade might profitably be introduced, but at the present time it is felt unwise to multiply grades. A classification under Grade IV has been given to certain tumors such as those illustrated in Figs. 7 to 10. These tumors show various structures ranging between adenocarcinoma and diffuse carcinoma; they may contain fairly uniformly small cells with small, round or spindle hyperchromatic nuclei, with an imperfect alveolar arrangement; they may consist of closely packed masses of tubulo-alveolar structures again with small cells, hyperchromatic nuclei and little stroma reaction. In the very diffuse type (Fig. 9) the carcinomatous structure may be determined with difficulty. The patient whose tumor is illustrated in Fig. 9 lived but five months from the onset of symptoms. Gelatinous adenocarcinomas characterized by diffuse infiltration with closely packed signet ring cells are likewise included in Grade IV. The above description indicates that the group as studied comprises a heterogeneous collection of tumors, no one of which has been met with sufficient frequency to estimate its prognosis as a single type.

Discussion

The distribution of tumors in the various grades was as follows: Grade I, 11 per cent; Grade II, 56 per cent; Grade III, 24 per cent; and Grade IV 9 per cent. The average age of incidence shows no marked difference in the various groups; it is slightly lower for Grade IV. The average age for Grades I, II, III is 58, 55, and 55 years respectively, whereas the average age for Grade IV is 47 years. It has been impossible to trace any relation between grade of tumor and location within the rectum. The average duration of symptoms previous to application for treatment of the rectal condition reveals certain grade differences; with Grade I it was twenty months, in Grades II and III, eleven and ten months, and in Grade IV, six months. Contrary to expectation no marked differences in average length of life were apparent in patients of the different age decades but with the same histological type of tumor.

Duration of Life: An attempt has been made to determine the length of life from the onset of symptoms to death with the four

tumor grades. This of course cannot be made with any marked degree of accuracy. Often the early symptoms are vague; a long history of hemorrhoids frequently confuses the picture and one is unable to decide when symptoms referable to some benign rectal condition give place to those of rectal cancer. The same objection of course applies to the analyses of age of incidence and duration of symptoms reported above. Duration cannot be accurately determined, but grade differences are sufficiently marked to merit attention. In cases dying, and presumably dying of the disease whether from metastases, hemorrhage, or infection, the duration of disease from onset to death was as follows: in Grade I, seventy months; in Grade II, thirty months; in Grade III, twenty-one months, and in Grade IV, ten months. In evaluating these groups, long operative survivals were eliminated; cases comprising the material had no surgical treatment other than colostomy. All had received more or less palliative radiation. The statistics offered are admittedly only approximations to the natural history of the disease.

Metastases: It is of interest to inquire what percentage of cases of the various grades had metastases; this again may not be answered with any degree of accuracy. For the determination of metastases we have only data derived from surgical examinations made at the time of colostomy. For this reason our figures show a considerably smaller number of cases with regional or distant metastases shortly after admission for treatment, than do those of Rankin and Broders. We admit the likelihood of their estimates. No case of Grade I tumor had metastasized at the time of colostomy; 10 per cent of Grade II tumors, 25 per cent of Grade III, and 60 per cent of Grade IV tumors had regional or distant metastases determinable at colostomy.

Change of Grade: The question of change in tumor grade during the course of the disease is of some interest. We have been able to study forty-six cases where more than one biopsy specimen has been obtained. Of these thirty-seven had two biopsies, eight had three, and one had four. In a total of 102 biopsies but ten grade changes were made; all but one of these consisted in an advance of the grade. In one case Grade II was changed to Grade I; in four cases Grade I was advanced to Grade II; in three instances Grade II was advanced to Grade III and in two cases the grade was altered from II to III and IV. It is safe to state that the tendency of a tumor of the rectum

is to maintain its type locally, rather than to develop progressively more malignant features. Again it must be emphasized that we are dealing with superficial biopsies and not the entire local tumor.

Prognosis: It is believed that the prognosis of rectal carcinomas with colloid or gelatinous features is somewhat better than in those cases where such characters are lacking. We have followed nineteen such cases, and have endeavored to grade the tumors from the appearance of the epithelial structures alone, neglecting the gelatinous elements. Unfortunately only in Grades III and IV may one draw any conclusion as yet. Of seven cases graded III, all are dead; the average duration of life from onset of symptoms to death was seventeen months. Of four cases in Grade IV, three are dead, the duration of the disease averaging thirteen months. The higher grades are restricted to those tumors containing large numbers of signet ring cells (Fig. 10). Despite this high mortality in the advanced grades certain operative results which we shall not attempt to summarize encourage the belief that the lower tumor grades of colloid carcinoma may run a very slow, relatively benign course, suggesting the value of surgery in this group even when tumors are recurrent.

Very little may be stated regarding those cases where marked discrepancy occurred between the estimated and the actual course of the disease. Almost all deaths took place outside of the hospital and the circumstances surrounding the deaths are not ascertainable. Local infection of tumors with resultant cachexia, hemorrhage; invasion of the bladder with resultant secondary urinary disturbances undoubtedly play a considerable rôle but occasionally one is at a loss to explain large differences between the estimated and the actual duration of disease. Almost all cases received radiation; as yet this factor's influence is not determinable.

Conclusions

1. The histological grading of rectal carcinomas in accordance with the suggestions of Rankin and Broders is of value in estimating the probable course of the disease.

2. Small biopsy specimens are sufficient for such grading.

3. Repeated biopsies do not show that change in type occurs sufficiently often in the course of the disease to interfere seriously with the prognosis suggested by the grade given at the time of the first biopsy.

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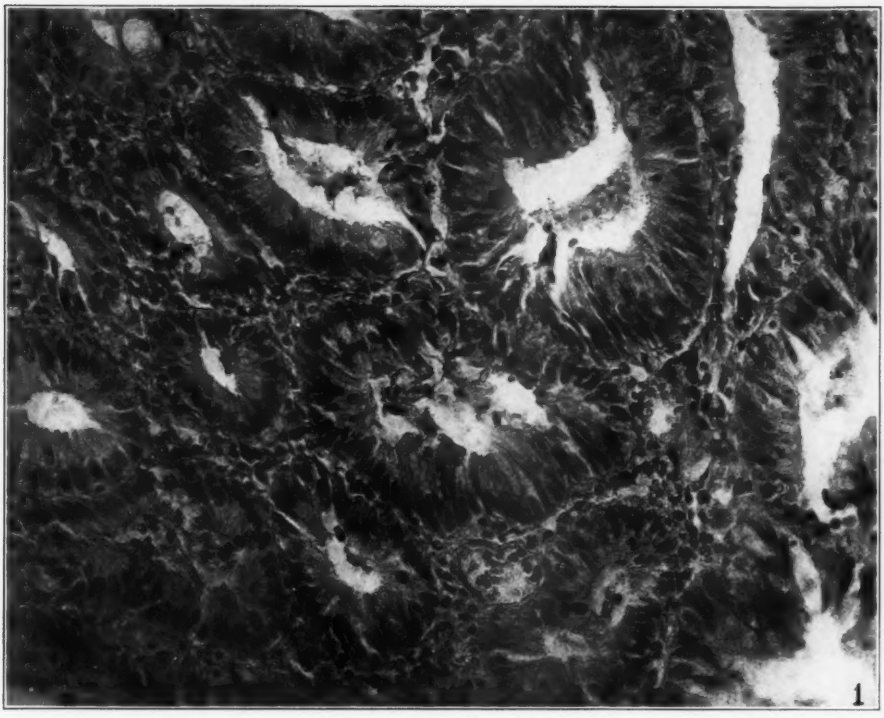
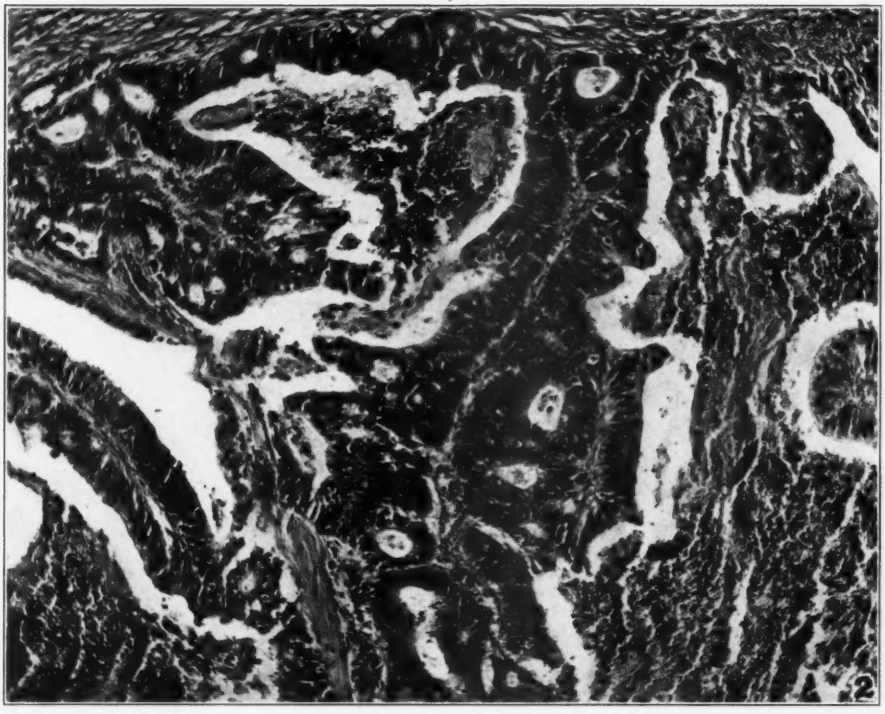
DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Adenoma destruens, Grade I.

FIG. 2. Adenoma destruens, Grade II.



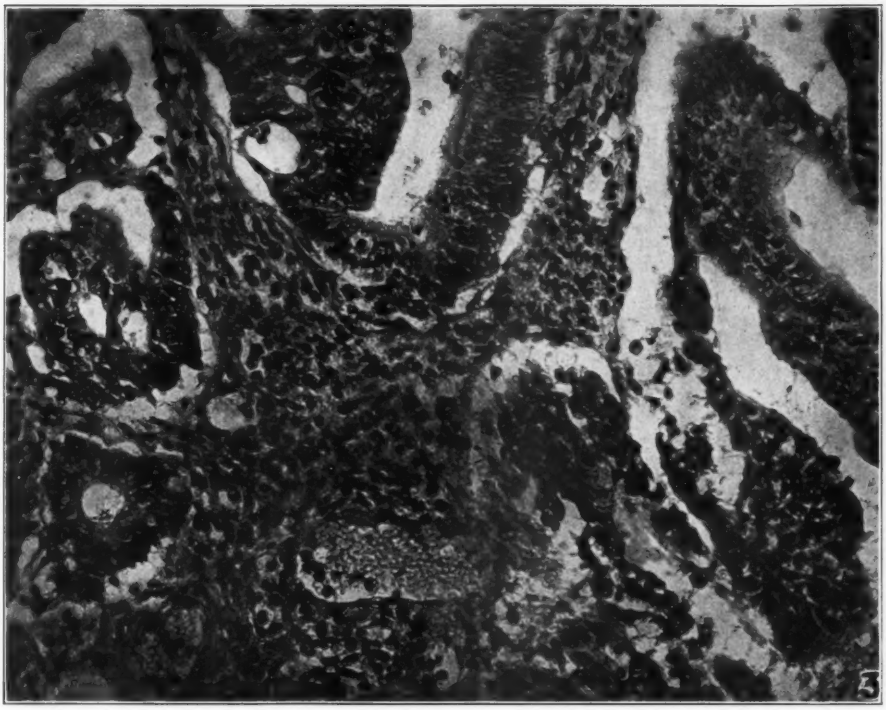


Stewart and Spies Histology in Grading of Rectal Carcinoma

Plate 25

Fig. 3. Adenocarcinoma, Grade III.

Fig. 4. Adenocarcinoma, Grade III.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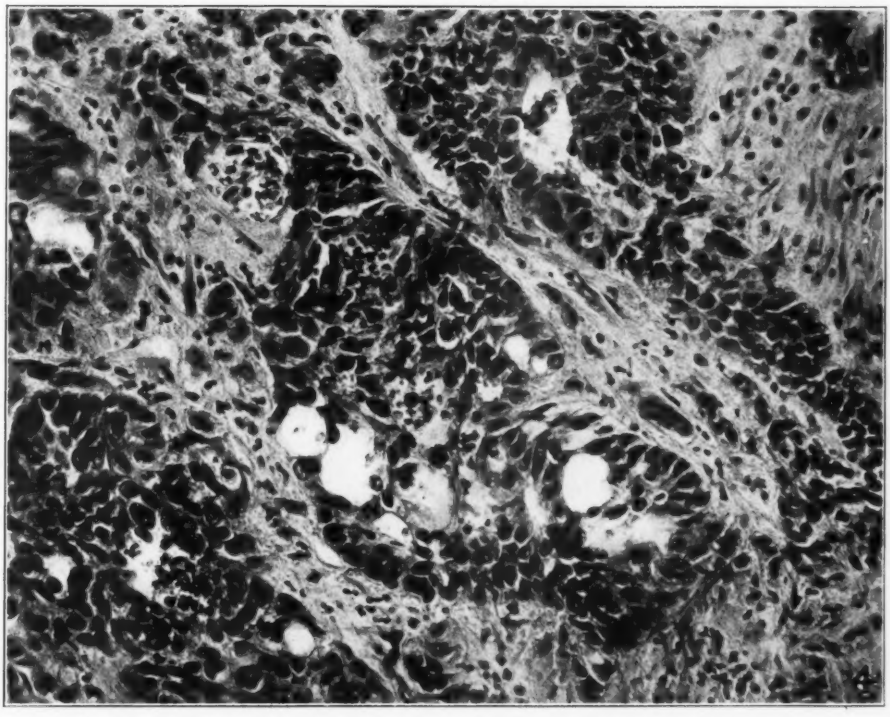
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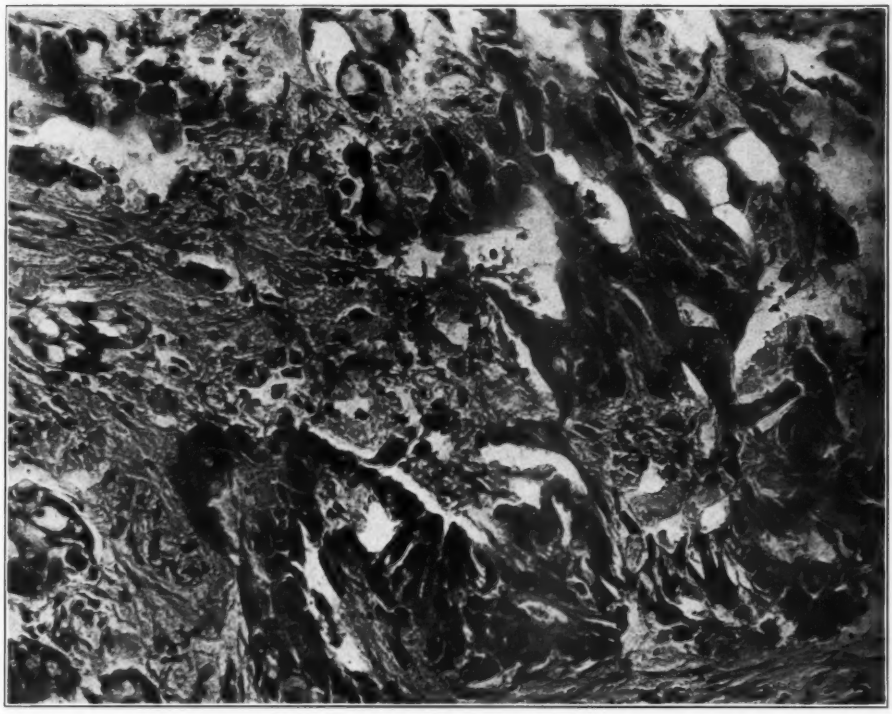
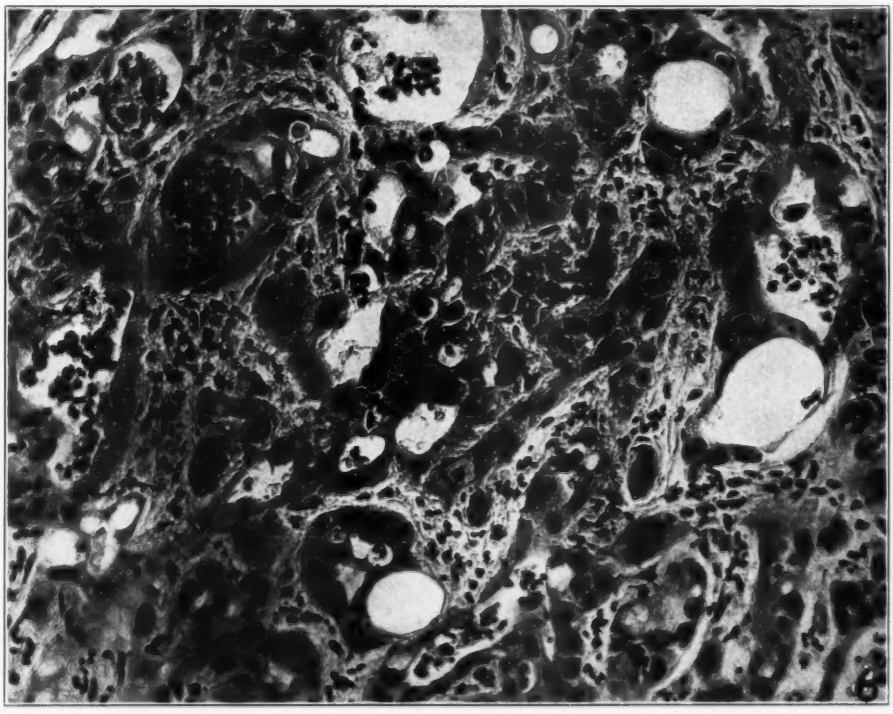
PLATE 26

FIG. 5. Adenocarcinoma, Grade III, graded as IV on account of infection.

FIG. 6. Adenocarcinoma, Grade III, advanced to Grade IV on account of infection.



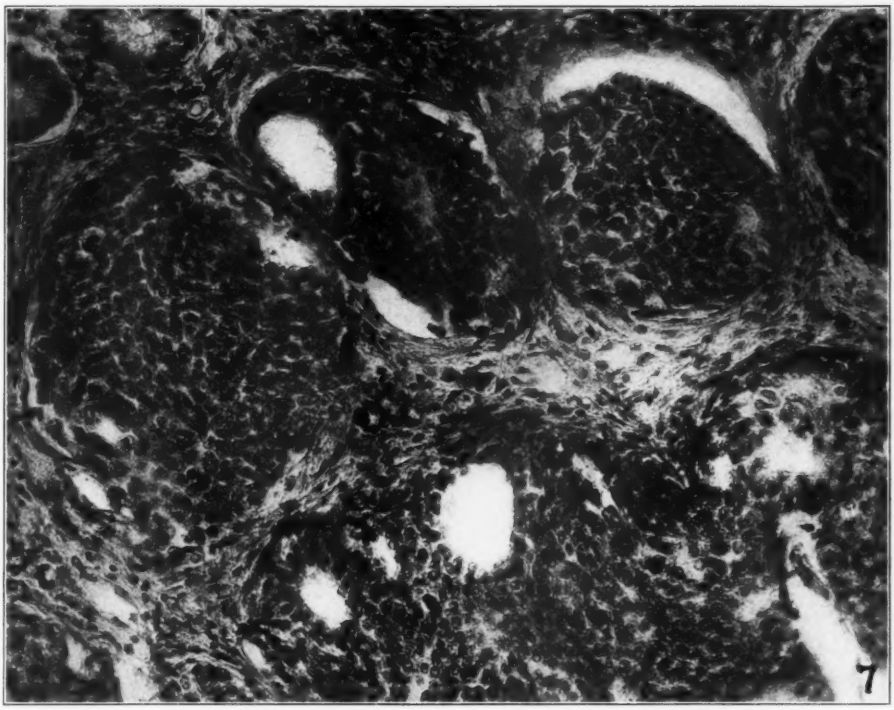


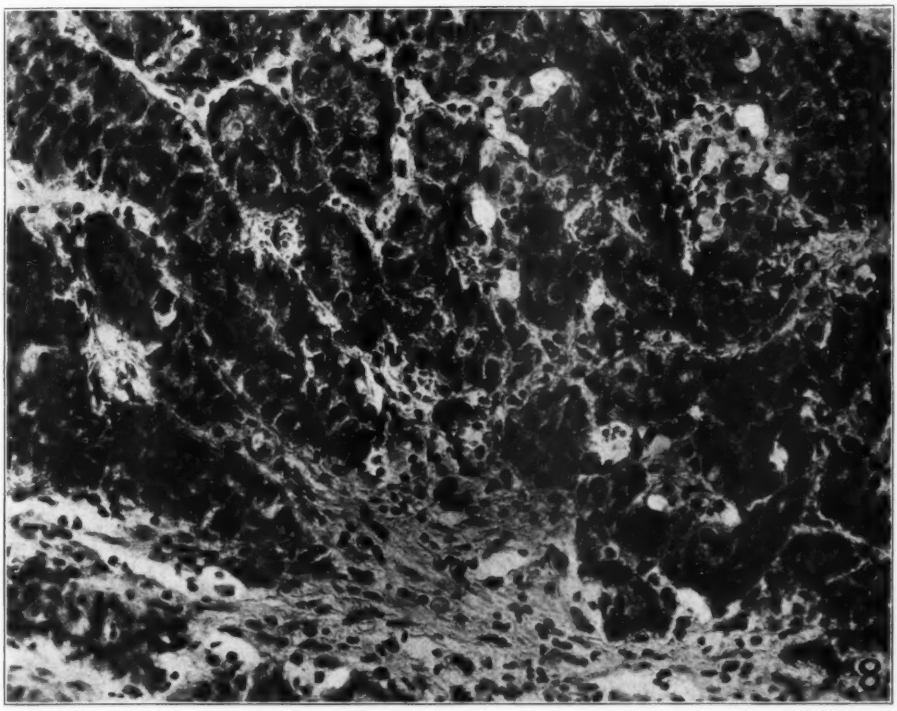
Steward and Spies Histology in Grading of Rectal Carcinoma

PLATE 27

FIG. 7. Adenocarcinoma, Grade IV.

FIG. 8. Adenocarcinoma, Grade IV.



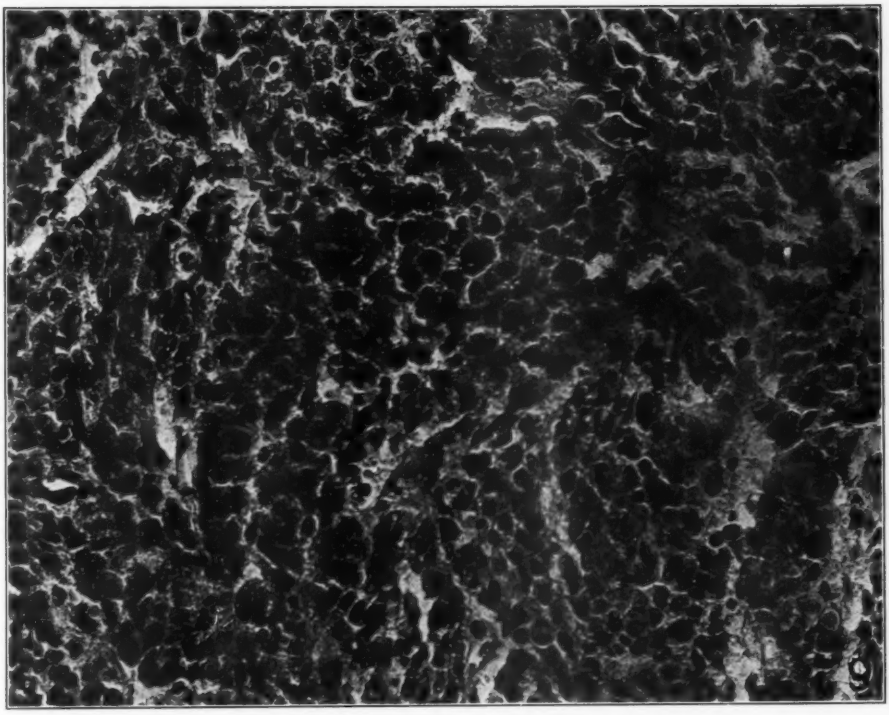


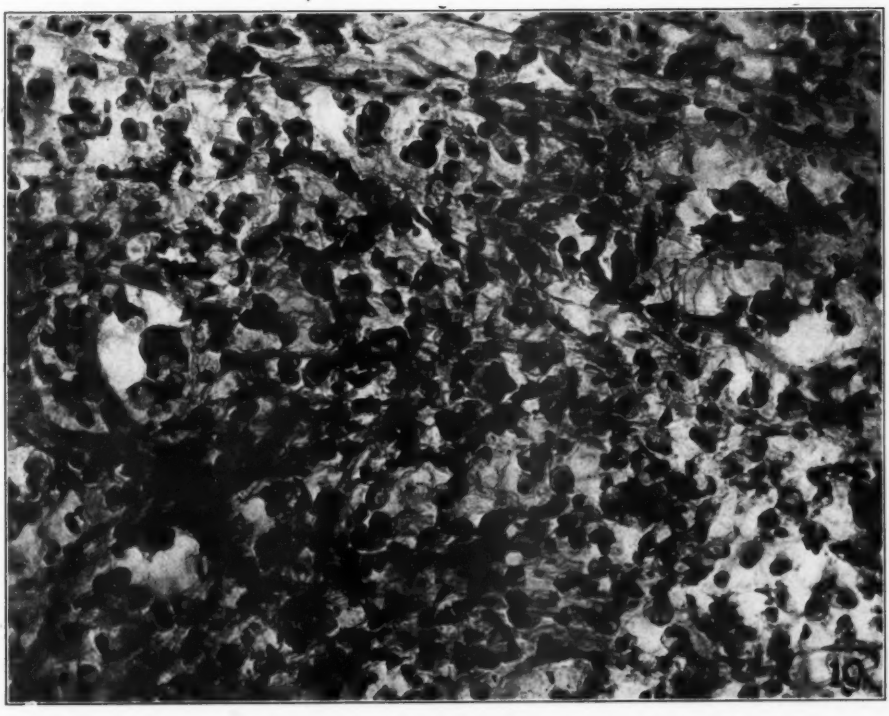
Stewart and Spies Histology in Grading of Rectal Carcinoma

PLATE 28

FIG. 9. Diffuse carcinoma, Grade IV.

FIG. 10. Gelatinous carcinoma with signet ring cells, Grade IV.





Stewart and Spies Histology in Grading of Rectal Carcinoma

THE PROGRESSIVE ANEMIA FOLLOWING A SINGLE INTRA-MARROW INJECTION OF B. WELCHII TOXINS *

JOHN C. TORREY AND MORTON C. KAHN

(From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York, N. Y.)

This study is in continuation of our investigation of the anemia produced in laboratory animals by a potent *B. welchii* toxin. In a previous communication attention has been directed to the consistently and abnormally high *B. welchii* counts in stools from pernicious anemia cases.[1] These findings have received confirmation in recent papers by Davidson,[2, 3] although Nye[4] believes that the difference from the normal as regards the number of *B. welchii* in the colon is more apparent than real. It was also shown[5] that a transitory anemia with a blood picture strongly suggestive of the pernicious type in a number of features could be produced in monkeys by intravenous inoculations of such a toxin. Similar observations for rabbits have also been made subsequently by Patterson and Kast,[6, 7] and Barach and Draper,[8, 9] although these investigators consider the resulting anemia of a secondary rather than primary type. The anemia produced by actual infection of rabbits with whole cultures of *B. welchii* was earlier studied by Cornell[10, 11] and by Reed, Orr and Burleigh,[12] and its pernicious-like features noted. It has been our experience, however, that the morphological and tinctorial blood changes in rabbits following intravenous inoculation of toxin, while somewhat suggestive of pernicious anemia, are not so striking as in monkeys. We have also reported[13] evidence of the absorption of this toxin from the gastro-intestinal tract of monkeys, the mucous membrane of which had been treated with a weak solution of sodium fluoride to induce a catarrhal inflammation. A blood picture in every way similar to that produced by intravenous inoculation resulted, together with a severe toxic degeneration of the marrow tissue, affecting particularly the fat cells and erythrocytes. The

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fluoride treatment alone caused no such marrow injury and only a moderate degree of anemia. The marrows of the two toxin-treated animals, however, were examined following their death from the anemia and there were thus no observations on the intermediate stages of the degenerative process. To gain information in regard to the effect of this sterile toxin on bone marrow, a large number of experiments have since been carried out in which a single small dose has been introduced directly into the tibial marrow of a series of rabbits and of a monkey. Somewhat to our surprise, we found that such a single treatment had a progressively injurious effect causing a chronic anemia with very marked loss of weight and frequently a fatal issue. Aside from any consideration of the possibility of absorption of *B. welchii* toxin being the essential etiological factor in the production of human pernicious anemia, the results aroused our interest because of the finding that the single inoculation into the marrow of one bone caused degenerative changes in quite equal, and at times in greater, degree in marrows of bones far removed therefrom. In view of the fact that the intravenous inoculation of the same dosage or a series of increasing amounts of toxin would not cause marrow changes of this character or degree, it is difficult to explain the apparent whole involvement of the marrow system following toxin injury to a single bone marrow.

It has been found that the *B. welchii* is capable of producing two apparently distinct hemolysins, one of which is active both *in vitro* and *in vivo* and the other, the more potent, causes blood destruction *in vivo* but only to a slight degree or not at all *in vitro*. Comparative tests of the action of these two products on bone marrow have been carried out both when introduced directly into the marrow and into the blood.

Technic

Toxin: The *B. welchii* toxin was prepared by the growth of the organism in casein-digest broth in sealed tubes as described heretofore.[2] We have found that 2 cc. of fresh rabbit blood per 30 cc. of medium may be substituted for fresh pigeon muscle. The toxin obtained is fully as potent and the *in vitro* hemolytic element is completely, or almost completely, absorbed during the growth period. This product will be designated as "toxin" in distinction from "hemolysin" which was obtained by growth of *B. welchii* in tubes

with the glass unsealed and in medium without addition of pigeon muscle or blood. Both products were rendered sterile by passage through Berkefeld filters. The toxin was tested for potency by injection in the breast muscle of pigeons. A good product caused death in 0.25 to 0.5 cc. dosage, in twenty-four hours.

Intramarrow Inoculation: The animal was placed under ether anesthesia, the hair shaved from a site over the outer posterior aspect of the left tibia about 2 cm. from the distal end and iodine applied. The skin was then stretched well over to one side and an incision, about 1 cm. in length, was made over the proposed point of inoculation. This was done so that when the skin was allowed to return to its normal position the puncture in the bone would be entirely covered and the danger of infection reduced to a minimum. The bone, almost directly under the skin, was laid bare and a small hole drilled of a diameter such that the hypodermic needle would fit snugly. This close fit is very important as otherwise the substance inoculated will not run into the marrow. The injection was made slowly, the usual dosage being 0.5 cc., and a few seconds allowed to elapse before the needle was removed. When properly carried out there was no leakage. With removal of the needle the skin was allowed to return to its normal position and a dressing applied. In no instance was there subsequently any evidence of infection and frequently when the animal was killed several weeks later the healing of the bone was so complete that the site of the puncture could not be found.

Experimental Results

The blood and marrow changes following the above treatment will be given first in detail in the case of a rabbit and a monkey and following that confirmatory tests will be described including a comparison of the action of *B. welchii* "hemolysin" and "toxin" introduced intravenously and into the marrow.

Rabbit No. 254: Male, weight 2200 gm. Inoculated into the left tibia with a single injection of 0.5 cc. of sterile "toxin" on March 11, 1926. The animal showed no obvious signs of discomfort following the injection. He was found dead seventy-five days later with a loss of weight of 580 gm., as shown in Table I. Twenty-four hours after the injection, both the hemoglobin and red cell count was reduced over 50 per cent. There was apparently also a marked leucocytosis

but this may have been a technical error as it was not noted in other rabbits similarly treated. About ten days following the inoculation the color index rose above 1.0 and stayed there until about two weeks before the death of the animal. For sixty days the animal was in a state of pronounced anemia with hemoglobin ranging from 50 to 60 and red cell count from 1,700,000 to 2,600,000. About one week before death a marked drop in hemoglobin occurred reaching a low point of 30. There was some evidence of a leucopenia, the white cell count reaching a minimum of 3,200 on two occasions. Morphological and staining changes in the blood were on the whole not quite so pronounced as reported heretofore as following intravenous toxin inoculations, although about five weeks after the toxin treatment poikilocytosis became very marked, much more so than when the toxin was injected intravenously. Nucleated red cells, punctated basophiles or those containing Howell-Jolly bodies were seldom observed. Pronounced anisocytosis was noted twenty-four hours following the injection and by the fourth day the microscopic fields showed principally microcytes staining more bluish with the Wright stain than normally. At least three attempts at regeneration are discernible; on March 19, April 10 and May 10. At these times there was a slight rise in hemoglobin or erythrocyte count and the blood picture was more nearly normal. On the day before death there was very marked anisocytosis, poikilocytosis, achromia and polychromatophilia. Two nucleated erythrocytes, three myelocytes and one myeloblast were found per 100 leucocytes counted.

For all the reports on the microscopic findings in the marrows and other tissues in this and other animals covered in this study, we are one indebted to Dr. James Ewing.

The marrows of the left and right tibia, right femur and left humerus were a dark maroon color throughout in the case of the two latter, and of a lighter shade in the distal halves of the two tibias. Sections of marrows from the left and right tibias, the left femur and left humerus showed essentially the same changes. Part of this tissue had been fixed in 10 per cent formalin and part in Zenker's fluid.* Dr. Ewing's report was as follows:

"There is in all a replacement of fat and lymphoid cells by a peculiar homogeneous granular material which appears to replace

* Our appreciative thanks are due to Dr. W. B. Castle of Boston City Hospital, who had sections of some of the Zenker-fixed material prepared.

TABLE I

Single Inoculation of B. Welchii Toxin into the Bone Marrow of a Rabbit

Rabbit No 254. Mature, Male. Weight 2200 gm.

Date	Toxin inoculation	Hemoglobin	Color index	Red blood count	White blood count	Differential white cell count							Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Howell Jolly bodies
						Polymorphonuclears	Lymphocytes	Basophiles	Large mononuclears	Eosinophiles	Transitional	Myelocytes					
1926		*per cent*															
Mar. 11	Normal	82		4,310,000	7,000	2	75	–	2	20	1	–	–	–	–	–	–
" 12	0.5 cc.	40	1.0	2,080,000	24,000	3	77	–	–	20	–	–	+++	–	1*	++	–
" 15	*B. welchii*	50	0.7	3,080,000	5,800	1	89	–	2	8	–	–	++++	–	–	–	–
" 19	toxin into	62	1.1	2,690,000	6,900	3	86	–	–	11	–	–	++	–	–	–	–
" 22	tibial	60	1.4	2,130,000	5,000	2	85	–	4	9	–	2	++	–	–	–	–
" 25	marrow on	55	1.4	1,910,000	3,200	–	72	–	1	26	1	–	+++	–	–	–	–
" 30	Mar. 11	51	1.4	2,150,000	5,200	–	78	–	–	21	1	–	–	–	–	–	–
Apr. 3		59	1.1	2,630,000	3,200	–	84	–	1	13	2	–	+++	–	–	–	–
" 10		61	1.4	2,100,000	5,000	–	73	–	1	25	1	–	–	–	–	–	–
" 17		58	1.3	2,240,000	2,400	4	72	3	–	21	3	1	–	++++	–	–	–
" 26		57	1.7	1,770,000	4,200	–	72	–	2	25	1	–	++++	++++	–	–	++
May 3		50	1.3	1,860,000	3,200	–	60	–	4	35	1	1	++++	++++	–	–	++
" 10		56	1.0	2,770,000	4,600	–	78	–	3	19	–	2	+++	+++	–	–	–
" 17		35	0.8	2,080,000	5,800	–	–	–	–	–	–	–	–	–	–	–	–
" 24		30	1.0	1,520,000	2,400	–	81	–	2	17	–	3	++++	++++	2	–	–

May 25 Rabbit died. Weight 1620 gm. at death.

* Per 100 leucocytes.

these cells and compose four-fifths of the tissue. The few remaining cells are mostly rather large, hyperchromatic granular mononuclears of the myelocyte type. No definite traces of forming red cells could be found. The changes resemble the so-called myeloblastic degeneration of bone marrow in pernicious anemia in an advanced atrophic stage." These findings are illustrated in Figs. 1 and 2. It may be noted that the degree of degeneration is even more marked in the right tibial marrow than in the left, into which the toxin had been injected.

Monkey Q: Mature, Nicaraguan Bull Head, Cebus type, female. May 6, 1926, 0.5 cc., *B. welchii* toxin inoculated into right tibia. A progressive anemia developed. When killed seventy-seven days later the animal was very anemic. The erythrocyte count was 1,700,000, the hemoglobin, 40. The tongue was pale and smooth, the liver, a light yellow reddish brown, the spleen, not enlarged and a light pink. No obvious lesions in the kidneys. The intestine was practically free of parasitic worms. The bone marrows were a dark maroon throughout, with the exception of those of the tibias in which the distal ends were of a lighter color. In this animal and in all *B. welchii* inoculated rabbits the blood serum was either colorless or a light straw color and never showed the lemon yellow hue so characteristic of pernicious anemia. The details of the blood examinations are shown in Table II. It will be noted that the erythrocytes fluctuated around 2,000,000 with counts below that mark during the last three weeks and the hemoglobin between 40 and 60 with evidences now and then of attempts at regeneration. The color index was generally slightly above 1.0 and there was at times a moderate leucopenia. Marked anisocytosis with many macrocytes was noted at practically every examination with occasionally distinct poikilocytosis and polychromatophilia. Occasional nucleated reds and myeloblasts were seen and Howell-Jolly bodies were considerably more prominent than in the blood of the rabbits similarly treated. In general features, however, the findings were very similar to those for the rabbits.

Microscopic examination of marrows of the right and left tibias, a femur and humerus showed degenerative changes, perhaps more marked in the right tibia into which the toxin had been inoculated, but prominent in the other marrows. The right tibia showed very numerous fat cells with scanty marrow cells which lay in clusters

TABLE II

Single Inoculation of B. Welchii Toxin into the Bone Marrow of a Monkey

Monkey Q. Young Adult Female

Date	Toxin inoculation	Hemoglobin	Color index	Red blood count	White blood count	Differential white count							Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Howell Jolly bodies
						Polymorphonuclears	Lymphocytes	Basophiles	Large mononuclears	Eosinophiles	Transitional	Myelocytes					
1926		*per cent*															
May 5	Normal	78		4,250,000	8,800	57	36	–	2	3	2	–	–	–	–	–	–
" 6	Normal	74		4,780,000	9,600	62	32	–	2	3	1	–	–	–	–	–	–
" 6	0.5 cc.																
" 10	B. welchii	42	1.05	2,030,000	5,000	26	69	–	–	3	2	++	+	–	1*	–	–
" 17	toxin in	45	0.9	2,320,000	5,000	37	59	–	1	1	2	–	++++	++++	–	–	–
" 24	tibia on	46	1.0	2,190,000	8,200	42	50	–	3	2	3	–	++++	–	–	–	++
June 2	May 6	41	1.0	2,090,000	11,200	49	39	–	1	8	3	–	++++	++++	2	–	–
" 9		57	1.4	2,070,000	6,600	36	51	–	2	8	3	–	++	++	–	–	–
" 16		53	1.2	2,200,000	8,400	24	67	–	2	5	2	–	+++	++	1	–	++
" 23		49	1.1	2,110,000	7,000	26	68	–	2	3	1	–	++	–	–	–	++
" 30		35	0.9	1,850,000	6,400	26	68	–	2	1	3	–	++++	–	1	–	–
July 8		42	1.2	1,710,000	7,000	22	72	–	1	3	2	–	++++	–	1	–	–
" 14		39	1.3	1,470,000	7,800	42	44	–	1	11	3	–	++++	–	–	–	–
" 21		40	1.1	1,700,000	5,600	25	72	–	2	1	–	–	++	–	–	–	–
July 22	Monkey very weak. Chloroformed in order to obtain organs in fresh condition.																

* Per 100 leucocytes.

between abundant fat cells. Giant cells were frequent. There was a great abundance of large phagocytic cells containing yellowish pigment. Many polymorphonuclear leucocytes were present throughout the tissue. No islands of normoblasts but many nucleated erythrocytes of about normal or slightly increased size were found. The general impression was of blood destruction with active regeneration of an irregular type. Local inflammation was indicated by the leucocytosis. The marrow of the left tibia was more cellular, fat cells less numerous and most of the cells lymphoid mononuclears. Comparatively few nucleated reds could be made out and there were few polymorphonuclear leucocytes (Fig. 8). The marrows of the right femur and right humerus were very cellular with practically no fat cells present and no islands of red cells. Most of the cells were lymphoid mononuclears. The findings in this monkey, accordingly, differed from those in the rabbit in that in the former the marrows did not show a replacement of the marrow and fat cells by the homogeneous granular material which was such a prominent feature in the rabbit marrows but these cellular elements were replaced by lymphoid cells. As with the rabbit, however, there occurred a very marked decrease in fat cells in marrows far removed from the site of inoculation. In both species there was evidence of marked degeneration of the blood-forming cells in the marrows *throughout* the body.

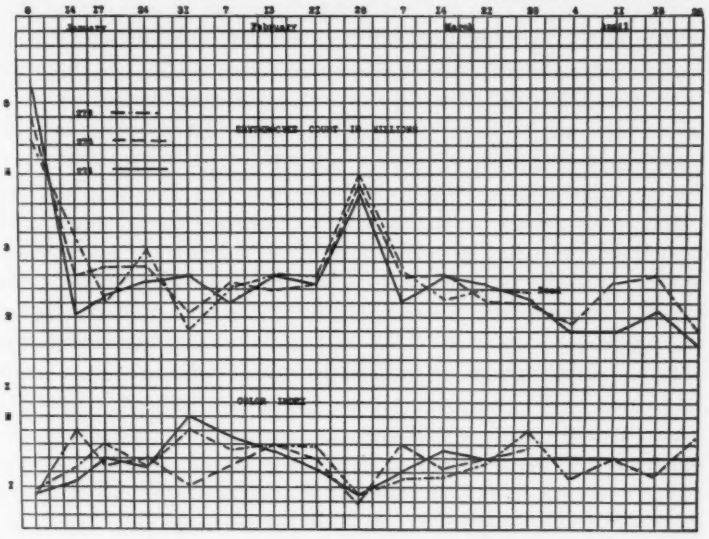
Confirmatory Tests

On January 13, 1927, three large rabbits, Nos. 272, 273 and 274, ranging in weight from 2000 to 2200 gm. were each inoculated into the left tibial marrow with 0.5 cc. of *B. welchii* toxin. This was a minimal lethal dose for a pigeon when injected into the breast muscle. One of the animals, No. 272, died seventy-four days later, repeating almost exactly the history of Rabbit 254 (death in seventy-five days). The erythrocyte count was 2,360,000 and the hemoglobin 76. Rabbit 273 was killed 102 days later in a very weak and anemic condition and without doubt would have died from its condition. The erythrocyte count was 1,830,000 and the hemoglobin 64. Rabbit 274 was in a marked to profound anemic condition for 112 days when he was killed in order to obtain the bone marrow. The erythrocyte count about that time was 1,600,000 and the hemoglobin 46. The morphological changes in the blood in all these three

rabbits were much the same as for Rabbit 254 (Table I) except that nucleated reds were not observed at any examination. Several inoculations of toxin, given intravenously in monkeys, cause after two weeks a flood of nucleated reds as described in a previous paper [2] but not when a single injection is given into the marrow. Polychromatophilia and basophilia in a moderate to a marked degree was

CHART I

Blood counts on rabbits after a single intratibial (marrow) injection of B. welchii toxin



observed at nearly every examination of the three rabbits beginning with the day following the inoculation. Transitional leucocytes and myelocytes were observed only on one or two occasions in each of these rabbits.

Chart I shows in detail the changes and fluctuations in the weekly erythrocyte counts and color indices determinations for the three rabbits. These curves have been superimposed and bring out in a striking way the almost identical reaction of the three animals to the single intramarrow inoculation of the toxin, both in degree and in point of time. The erythrocyte count dropped to between 2,000,000 and 3,000,000 the day following the inoculation and remained at that level continuously for seven weeks. There then occurred in all three animals a strong attempt at blood regeneration, as in each

the erythrocyte count rose to nearly 4,000,000 and the color index dropped to below 1.0. This lasted only a few days at the most, for on the eighth week examination the red cell count had dropped to the former level and the color indices were considerably above 1.0, as had been the case heretofore. In the case of Rabbit 254 a similar major regenerative attempt occurred in the eighth week (Table I). Minor remissions may also be noted in the third to fourth weeks and in the thirteenth to fourteenth weeks in the two surviving that long.

Rabbit 272 at death was markedly emaciated, the loss in weight being about 41 per cent. Rabbit 273, however, showed a loss of only 3 per cent. The internal organs in all were anemic but showed nothing of particular note macroscopically except a shrunken condition of the spleens. In two normal rabbits of approximately the same size, the spleens averaged 8 by 60 mm., whereas in Rabbit 272 the measurement was 5 by 15; in No. 273, 4 by 46; and in No. 274, 5 by 45. Microscopic examination showed heavy pigmentation and large microphages in the spleen of No. 272. The liver of No. 272 exhibited marked congestion with a rich deposit of fine granular yellowish pigment within the liver cells but no fatty or granular degeneration, whereas the liver of No. 273 was extremely fatty with acute universal lipoid degeneration and occlusion of capillaries from swollen liver cells. The marrows of Nos. 272 and 273 showed, in general, similar changes to those already noted for Rabbit 254, both macroscopically and microscopically. In No. 272 the changes in the right and left tibial marrow (into which the toxin was injected) were the same in type and degree, consisting of a remarkable proliferation of large cells replacing fat and causing an extensive atrophy of the blood forming cells. Among the remaining cells there were many polymorphonuclear leucocytes, a few myelocytes and many large hemoglobin-holding cells with pyknotic nuclei. There were also many pigmented microphages. The total blood-producing capacity of these marrows was greatly reduced and the type of cell formation abnormal. The right and left femurs showed similar changes but less marked. In No. 273, which lived longer than No. 272, the marrow changes in the right tibia were the same as in the left but less marked as the marrow was much more cellular and the large clear cells were less numerous. The changes in the right and left femurs were of the same character as in the left tibia but less marked.

In an attempt to determine the rapidity of the toxin action on the

bone marrows, Rabbit 500 was inoculated with 0.5 cc. *B. welchii* toxin into the left tibial marrow and then killed after eighteen hours. Before inoculation the erythrocyte count was 4,600,000 and the hemoglobin 80. Eighteen hours following inoculation the erythrocytes were 2,250,000 with marked anisocytosis and polychromophilia and slight poikilocytosis, the hemoglobin 59, color index 1.3, leucocytes 8,800. There was some hemorrhage into the left marrow. Microscopically there were the same changes in the right and left tibial marrows but curiously more marked in the right than the left. The right tibia (on the other side of the body from the inoculation) showed the tissue considerably damaged with complete atrophy of marrow cells in the center of the marrow, and marrow cells throughout much diminished in number. A good many fragmented nuclei, a great many polymorphonuclear leucocytes and numerous eosinophiles were noted; also cells showing atypical mitoses and degenerating giant cells. The fat cells were still normal and abundant. The right femur showed irregular congestion with areas devoid of cells, considerable degeneration of giant cells and many polymorphonuclear leucocytes. The left humerus exhibited the same changes as the femur with the same type of fat cells showing granular degeneration. The changes in the right tibial marrow are illustrated in Fig. 2, which may be compared with the normal marrow as shown in Fig. 1. The photographs reproduced in the plates are all from the same general region of the tibial marrow, about 1 cm. from the proximal end.

The above observations indicate that within the first twenty-four hours following an intratibial inoculation of the toxin the damage is by no means confined to the marrow into which the inoculation was made but has caused marked blood destruction, to the same degree as if the inoculation had been made intravenously, and also degenerative changes in marrows far removed from that inoculated.

Series of Rabbits Killed and Examined Twelve Days after Inoculations

A rabbit (No. 98) was inoculated into the left tibia with 0.5 cc. of toxin, killed after twelve days and the marrows examined to determine the changes occurring after that interval. Twenty-four hours after the injection, examination of the blood showed a severe condition of anemia. There was an extreme degree of anisocytosis with a strong predominance of dark microcytes, marked poikilocytosis,

many shadow erythrocytes with cell walls broken and also numerous nucleated forms. The animal was so anemic that it was only with difficulty that sufficient blood for a count was obtained from a peripheral vessel. Further details are shown in Table III. The blood destruction twenty-four hours following the intramarrow injection was far more marked than that shown after the same period by Rabbit 593, inoculated intravenously with the same dosage of toxin (See Table IV). Rabbit 98, when killed after twelve days, had decreased in weight from 1820 to 1170 gm., a loss of 650 gm. or somewhat over one-third. Autopsy did not show anything of note except a rather small spleen, 35 by 8 by 4 mm. The marrows were a bright red throughout. Microscopic examination of the right tibial marrow showed an extremely marked atrophy of marrow cells and a marked increase of fat cells, most of which had degenerated. Polymorphonuclears were extremely abundant. The same changes were observed in the other marrows examined, *viz.*, the left tibia and right and left femurs. Degenerative changes were thus well advanced at this time (Fig. 3). The blood examination of this rabbit after the twelve-day period is detailed in Table III and for comparison the findings for a rabbit (No. 593) which was given a single inoculation of the same amount, 0.5 cc., intravenously and killed after twelve days (Table IV). The erythrocyte count for this latter animal showed a drop from 4,840,000 to 3,230,000 but no change in hemoglobin. There was a moderate degree of anisocytosis, slight polychromatophilia but the blood injury was in no way comparable to that of No. 98, as is shown in Table IV. The report on the microscopic examination of the marrows of this rabbit (No. 593) is as follows:

Right Tibia: The marrow is rather cellular, with fat and cellular tissue about equal. The fat cells show mucous degeneration in moderate degree. Polymorphonuclear leucocytes are very numerous. There are a great many large mononuclear acidophile cells and many of these appear to be megaloblasts. The blood formation seems to be of megaloblastic type (Fig. 3).

Right Femur: The marrow cells are limited to a narrow ring about the periphery of the marrow. There is slight mucous degeneration of fat cells with considerable capillary congestion. The polymorphonuclear cells are rather numerous. There are an unusual number of very small pyknotic nuclei which seem to belong to nucleated reds.

TABLE III

Comparative Effect of the Intramarrow (Tibia) Inoculation of B. Welchii Toxin, Hemolysin and the Sterile Medium. Animals Killed Twelve days Later and Marrows Examined

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1927		*per cent*								
No. 98	May 27	None	85	1.0	4,200,000	6,400	–	–	–	–	–
Wt. 1820 gm.	June 2	0.5 cc. Toxin									
	" 3		27	1.0	1,300,000	–	++++	+++	5	–	++++
	" 13		48	1.2	1,920,000	3,800	++++	++	–	–	++
Wt. 1170 gm.	" 14	Killed									
No. 96	May 27	None	86	1.0	4,370,000	6,400	–	–	–	–	–
Wt. 1780 gm.	June 2	0.5 cc. Hemolysin									
	" 3		55	1.1	2,360,000	6,400	+	–	–	–	–
	" 13		45	0.8	2,630,000	5,000	++	–	–	–	–
Wt. 1940 gm.	" 14	Found dead									
No. 97	May 27	None	85	1.0	4,260,000	6,000	–	–	–	–	–
Wt. 1880 gm.	June 2	0.5 cc. Sterile medium									
	" 3		76	1.0	3,600,000	7,100	–	–	–	–	–
	" 13		50	1.0	2,440,000	4,600	–	–	–	–	–
Wt. 1275 gm.	" 14	Killed									

There are many large acidophile cells and some of these are eosinophile and granular. There are some large nucleated reds.

Right Humerus: About half the marrow is cellular and half fat tissue. The cellular marrow is more cellular than in the other specimens. Polymorphonuclear and eosinophile cells are very numerous. There are also a great number of small pyknotic nuclei belonging to the series of normoblasts, but few large nucleated reds are seen. The fat cells show little change.

The changes were thus much more delicate than in Rabbit 98 and not of the same character, particularly as regards degeneration of the fat cells.

Rabbit 96 of this series received into the left tibial marrow 0.5 cc. of a growth product of *B. welchii* which was very strongly hemolytic but weakly toxic, as shown by inoculation into the breast muscle of a pigeon. As noted above, our "toxin" inoculated in 0.25 to 0.5 cc. dosage would kill the bird within twenty-four hours with the development of a local massive and hemorraghic edema, whereas 1 cc. of this hemolysin caused only very slight local reaction and no toxic symptoms. This hemolysin was prepared by growing *B. welchii* in the same medium as for the toxin (3 per cent peptone, 0.5 per cent glucose, casein digest broth) except that no pigeon muscle or fresh rabbit blood was added and the tube was not sealed in the blow flame. Further details in regard to the nature and antigenic properties of these two products will be given in another publication.

This rabbit was apparently in good condition eleven days after the injection of the hemolysin into the marrow. It had increased in weight from 1780 to 1940 gm. (compare with No. 98). Ten cc. of blood was taken from the ear vein. On the next day, however, the animal was found dead. Necropsy did not disclose the cause. The degree of anemia was not nearly as great as for No. 98. The spleen measured 44 by 11 by 3 mm., a little larger than that of No. 98. The marrows differed markedly from those of No. 98 in being a much darker red throughout. Microscopic examination of the left tibial marrow showed thrombosis of the main central vein with the surrounding tissues infarcted. There was also a very marked increase of large pale proliferating fat cells and a corresponding diminution of marrow cells which were not very much altered. There were many large hemoglobin-holding cells and eosinophiles. The marrow cells were not very much altered and there was not nearly as marked

TABLE IV

Blood Changes in Rabbit Twelve Days after a Single Intravenous Inoculation of B. Welchii Toxin

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1928		*per cent*								
No. 593	Jan. 26		85	0.88	4,840,000	9,200	tr.	–	–	–	tr.
Wt. 2960 gm.	Mar. 1	0.5 cc. Toxin intravenously									
Wt. 3240 gm.	" 13		84	1.4	3,230,000	6,400	++	–	–	–	+

atrophy as in the case of Rabbit 98, in which the marrows were far more damaged. The right femur, the side opposite the site of inoculation, showed similar changes with moderate atrophy of marrow cells and considerable proliferation of fat cells. In fact, the proliferation of large fat cells was a very striking feature in this animal, and the findings, in general, were markedly different from those in the rabbit (No. 98) inoculated with the toxin.

Rabbit 97 of this series was inoculated into the left tibia with 0.5 cc. of the sterile culture medium. Subsequent examination disclosed that the animal was not entirely normal as there was evidence of an old lesion in the right tibial marrow. The animal lost weight markedly during the twelve days, decreasing from 1800 to 1275 gm. There was also a marked decrease in the erythrocyte count and the hemoglobin, as shown in the table, but the color index remained almost exactly 1.0 and no abnormalities were observed in the stained blood films. At necropsy the spleen measured 39 by 7 by 4 mm. Other organs appeared normal. The marrows were a bright red throughout, except the distal ends of the tibias which are normally much lighter in color. The right tibia, the site of an old lesion, showed a marked proliferation of fat cells but the changes were much less marked than in No. 98. The right femur marrow showed no change and was normal in appearance.

By way of further control two rabbits were inoculated intramarrowly with the sterile culture medium and observed for a period of about nine weeks. Rabbit 579 was inoculated into the right tibial marrow with 0.5 cc. of the sterile broth medium in which pigeon muscle had not been soaked, and No. 587 with the broth to which the muscle was added and incubated over night. The details for these animals are given in Table V. It will be noted that at the end of the period both animals had gained in weight in contrast with the very marked loss generally observed in the animals inoculated in the same way with the toxin. The erythrocyte counts and the hemoglobin fluctuated with a slight downward tendency in No. 579, but remained quite constant with No. 587. The color indices remained close to 1.0 and the blood showed no morphological or staining abnormalities throughout the experiment. Both animals appeared in fine condition at the termination of the experiment. The report on the right and left tibia and the right femur marrows of these rabbits is as follows:

TABLE V

Control Rabbits. Effect of Intramarrow (Left Tibia) Inoculation of Sterile Culture Medium Without Pigeon Muscle (No. 579) and with Pigeon Muscle (No. 587)

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1928		*per cent*								
No. 579	Feb. 6	None	90	0.9	4,960,000	11,000	–	–	–	–	–
Wt. 2972 gm.	" 7	0.5 Sterile culture medium									
	" 14		85	1.0	4,010,000	7,000	–	–	–	–	–
	" 28		82	1.0	4,240,000	14,600	–	–	–	–	–
	Mar. 6		84	1.0	4,320,000	5,600	–	–	–	–	–
	" 13		82	1.0	4,270,000	7,000	–	–	–	–	–
	" 27		85	1.0	4,070,000	8,600	–	–	–	–	–
Wt. 3225 gm.	Apr. 10		81	1.0	4,080,000	4,200	–	–	–	–	–
No. 587	Jan. 26	None	85	0.9	4,850,000	6,200	–	–	–	–	–
Wt. 2675 gm.	Feb. 2	0.5 cc. Sterile culture medium*									
	" 9		88	0.9	4,910,000	8,200	–	–	–	–	–
	" 16		87	1.0	4,690,000	6,000	–	–	–	–	–
	" 23		87	1.0	4,500,000	6,600	–	–	–	–	–
	Mar. 1		82	1.0	3,950,000	7,200	–	–	–	–	–
	" 8		84	0.87	4,800,000	7,200	–	–	–	–	tr.
	" 13		78	1.1	3,520,000	8,400	–	–	–	–	tr.
	" 29		82	0.9	4,620,000	9,400	–	–	–	–	–
Wt. 3075 gm.	Apr. 12		89	1.0	4,470,000	9,800	–	–	–	–	–

* Sterile pigeon muscle was added to the medium and incubated overnight.

"There is a marked reduction of all types of cells. The fat cells are normal. The femur marrows are slightly more cellular than the tibial. There is no other change in the marrow except simple atrophy. The condition is probably physiological and preëxisting." The appearance of the right tibial marrow of Rabbit 579 is shown in Fig. 7. This cellular atrophy may possibly have been induced by the medium ingredients, particularly the peptone, but the change was not sufficient to affect the blood picture and was in no way comparable to that following the toxin.

It seems evident, then, that medium ingredients were not responsible for the progressive degeneration of the hematopoietic system following the intratibial inoculation of the *B. welchii* toxin.

In the case of one monkey it was determined that the immunity following a long series of intravenous inoculations of *B. welchii* toxin included also the marrow. This monkey (J) had received twenty-eight treatments between January 14, and March 12, 1926, in dosage ranging from 1 cc. to 20 cc. After a prolonged period of anemia the blood picture returned to approximately normal. The animal was allowed to rest for a month and was then given 1 cc. of *B. welchii* toxin into the tibial marrow. Blood examinations during the following two weeks indicated the treatment had no effect whatever and it was thus evident that the immunity produced by the intravenous inoculation extended to such a susceptible tissue as the bone marrow.

Intravenous Inoculation of *B. Welchii* Toxin and Hemolysin

For purposes of comparison with the effects of the intramarrow inoculations, a description is given of blood and marrow changes following the intravenous inoculation in rabbits with the same sterile *B. welchii* growth products. Three rabbits, Nos. 112, 114 and 269, were given five injections of 1 cc. dosage at weekly intervals and finally an intraperitoneal inoculation of 10 cc. Rabbit 112 received the "hemolysin," Rabbit 114 the toxin and Rabbit 269 a mixture of equal parts of the two. The toxin was only feebly hemolytic when tested *in vitro*, 1 cc. causing at the most partial lysis of 1 cc. washed (5 per cent) sheep cells after one and a half hours at 37° C., whereas the "hemolysin" prepared by growing the *B. welchii* in the same broth medium but without pigeon muscle or blood and with the tube

TABLE VI

Blood Changes in Rabbits Inoculated Intravenously with Sterile Toxic Products of B. Welchii Growth

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1927		*per cent*								
No. 112	Mar. 23	Hemolysin	92	0.97	4,710,000	10,800	–	–	–	–	–
Wt. 2,670 gm.	11.05 a.m.	1 cc.									
	4.05 p.m.		66	1.0	2,670,000	5,200	+	–	–	–	–
	Mar. 24		66	1.0	3,260,000	17,000	++++	–	–	–	–
	" 26		51	0.8	3,060,000		++++	–	–	–	–
	" 30	1 cc.	61	2.3	1,390,000	5,000	++	+	–	–	+++
	Apr. 6	1 cc.	69	1.0	2,710,000	5,500	++	–	–	–	+
	" 11	1 cc.	76	1.7	2,110,000	8,400	++	–	–	–	–
	" 20	1 cc.	64	1.7	1,940,000	11,000	+++	+	–	–	+++
	" 27	10 cc. Intraperit.	60	0.75	4,060,000	12,600	++	+	–	–	+++
Wt. 2,445 gm.	May 4		65	1.0	3,020,000	7,000	++	–	–	–	+
No. 114	Mar. 23	Toxin	92	0.97	4,740,000	9,200	–	–	–	–	–
Wt. 2,640 gm.	11.25 a.m.	1 cc.									
	4.25 p.m.		70	1.3	2,670,000	7,800	++	–	–	–	–
	Mar. 24		66	1.1	2,900,000	17,200	++++	–	–	–	–
	" 26		53	1.2	2,290,000		+++	–	–	–	–
	" 30	1 cc.	72	1.8	2,090,000	4,800	++++	++	–	–	+++
	Apr. 6	1 cc.	59	1.0	2,800,000	6,200	+++	+	–	+++	++
	" 11	1 cc.	79	2.1	1,890,000	3,000	+++	–	–	–	–
	" 20	1 cc.	74	1.7	2,160,000	7,200	+++	+	–	–	–
	" 27	10 cc. Intraperit.	56	0.72	3,990,000	10,600	+++	–	–	–	++
Wt. 2,440 gm.	May 4		57	1.0	2,660,000	4,800	++++	–	–	–	++++
No. 269	Mar. 23	Toxin-hemolysin	81	0.9	4,580,000	9,800	–	–	–	–	–
Wt. 2,610 gm.	11.50 a.m.	1 cc.									
	4.50 p.m.		61	1.6	1,870,000	11,200	–	–	–	–	–
	Mar. 24		55	1.0	2,750,000	10,200	++++	–	–	–	–
	" 26		50	0.9	2,730,000		+++	–	–	–	–
	" 30	1 cc.	56	3.0	910,000	4,000	++++	–	–	–	+++

unsealed, was very potent *in vitro* causing complete lysis of 1 cc. of the washed sheep cells in dosage of 0.05 to 0.025 cc. within one hour at 37° C. One week following the first inoculation the greatest amount of blood destruction was observed in No. 269, receiving the mixture. Comparing the toxin and the hemolysin, a somewhat greater degree of blood destruction was noted in one hour following the inoculations of the hemolysin (Rabbit 112) than the toxin (Rabbit 114); as shown particularly in the hemoglobin readings, Rabbit 112 hemoglobin decreasing from 92 to 66 while that of Rabbit 114 dropped only from 92 to 85. As shown in Table VI, however, this difference was more than made up after a lapse of a week, the toxin rabbit revealing then the greater degree of blood destruction but not so much as the toxin-hemolysin animal (No. 269). These differences were confirmed in another set of rabbits. In spite of repeated intravenous inoculations of these *B. welchii* products, a marked immunity developed in all within three to four weeks. This immunity was strong enough to protect the blood against the 10 cc. intraperitoneal inoculations in the toxin and hemolysin rabbits but not in the animal treated with the toxin-hemolysin mixture.

The marrows of Rabbits 114 and 269 were a dark maroon color, whereas those of No. 112 (hemolysin rabbit) were a lighter red. Microscopically the marrows of Nos. 112 and 114 did not show any definite changes from the normal except those of No. 112 which appeared somewhat more cellular.

The above animals had developed an immunity and it was quite possible that injury done to the marrows might have undergone repair. Accordingly another rabbit (No. 582) was given three intravenous inoculations of toxin within a period of twelve days and killed ten days after the last injection and while still in a markedly anemic condition, the erythrocyte count being 2,340,000 and the hemoglobin 60 with marked anisocytosis and polychromatophilia. The marrows were dark red. Sections of the right tibial marrow showed a very cellular and considerably congested condition, but there were no changes in the fat cells. All types of marrow cells appeared in normal numbers, with eosinophiles moderately numerous. There were no definite pathological changes. A description has already been given (page 131) of the findings in a rabbit (No. 593) after a single intravenous inoculation of toxin.

Discussion

The preceding experiments show clearly that a single inoculation of *B. welchii* toxin in small dosage into one bone marrow of a rabbit or a monkey causes a toxic degeneration of the cellular elements of the marrow with a consequent anemic condition persisting until the death of the animal ten weeks or more later (about 50 per cent of our rabbits died) or as long as the animal was observed, up to seventeen weeks. This degenerative change affects particularly the fat cells which seem to increase in number at first, but later undergo mucous degeneration to such a degree that they are finally entirely replaced by a peculiar homogeneous granular material. At the same time there occurs a degeneration of the marrow cells and the erythrocyte-forming cells. In the intermediate stages unusual numbers of polymorphonuclear neutrophiles and eosinophiles accumulate in the marrow. These changes begin immediately after the inoculation of the toxin and are not restricted to the marrow into which the injection is made but are quite as prominent or more so in marrows far removed therefrom, even within twenty-four hours or less following the treatment. Sufficient marrows were examined to indicate that the whole system was probably involved. Control inoculations with the sterile medium itself showed that the injury was due to the toxic growth products of the *B. welchii* and not to the medium ingredients. Such extreme marrow changes further do not follow intravenous inoculation of the toxin regardless of the dosage used or the number of inoculations. The marrows become more cellular and congested after intravenous inoculation. Following the first inoculation, the fat cells may show mucous degeneration in moderate degree but an immunity soon develops which arrests the process and permits a return to normal even though very large doses of toxin are employed (Table VI). It is reasonable to suppose that the inoculum reaches the various marrows of the body in the same or greater strength when the same dosage is injected directly into the blood stream as when inoculated into a single marrow as it is the blood and lymph which transports it in each case. If the degenerative changes were confined to the marrow receiving the toxin or were much greater there than in the marrows far removed, the severer effect would be expected because of contact with the undiluted toxin. Such, however, is by no means the case, as our observations demonstrate.

What then causes the strikingly different effect on the marrow system when the toxin is injected intravenously and intramarrowly? We have as yet no experimental data which would serve to explain the difference but the suggestion may be made that the undiluted toxin in its destructive action on the marrow tissue with which it is brought into direct contact may give rise to secondary toxic products particularly injurious to marrow tissue, and which on absorption throughout the lymph and blood are brought in contact with all the body marrows, causing as grave an injury to those farthest removed as to that into which the toxin was directly inoculated.

It would seem probable that an experimental anemia produced in this way may prove helpful in the study of the treatment of pernicious anemia, particularly as regards the use of liver extracts and their standardization. At our suggestion the methods employed in this study are now being used at the Lederle Antitoxin Laboratories in an experimental investigation of their applicability to liver extract standardization. In a preliminary report by Beard, Clark and Moses,[14] it is clearly demonstrated that liver extract feeding to rabbits with the chronic anemia produced in this way is beneficial, resulting in a prompt and marked rise in reticulocytes, hemoglobin and red cell counts. There seems also to be a proportionality between the amount of liver extract given and the increase in number of red cells and hemoglobin content. The benefit, in fact, seems to parallel that occurring in a pernicious anemia patient on liver extract. Their findings offer hope that a method for standardization of liver extracts may thus be established. Whipple [15] has found that a secondary anemia produced in dogs by repeated bleedings is only slightly benefited by feeding liver extract, although marked improvement follows the use of whole liver. It would seem, then, that our method offers a much more delicate method for potency titration. It is also suggested that this experimental anemia may provide a method for evaluating the blood regenerating potentialities of various chemical fractions of liver and other organs.

Summary

1. It has been found that the single inoculation of 0.5 cc. of a potent sterile *B. welchii* toxin into a tibial marrow of a rabbit or a monkey gives rise to a chronic, persistent and finally often fatal anemia characterized by low hemoglobin content of the blood, low

erythrocyte count and a color index generally above 1.0. Anisocytosis and at times poikilocytosis are pronounced.

2. A single intravenous inoculation of *B. welchii* toxin of the same dosage or a series of increasing doses gives rise to an anemia of the same type as that resulting from marrow inoculation but is followed within three to four weeks by an immunity and a return of the blood to normal or nearly normal condition. Such intravenous inoculation of the toxin causes some mucous degeneration of the fat cells and an increase in the cellular elements in the marrow, particularly the leucocytes, but results in nothing like the destructive action of inoculation directly into the marrow.

3. The single inoculation of 0.5 cc. of the toxin into a tibial marrow starts a degenerative process which apparently affects the whole marrow system. Definite evidence of beginning degenerative changes were noted in the marrows on the side of the body opposite the site of inoculation within eighteen hours. After twelve days the process was well advanced as shown by the marked mucous degeneration of the fat cells, great diminution in other normal marrow and blood-forming cells and a marked increase in polymorphonuclear leucocytes. In rabbits which had died eleven or more weeks after the inoculation the marrows were found to be in an advanced stage of degeneration with fat cells replaced by a granular material and an extreme atrophy of the cellular elements. Nearly all the animals showed, at whatever stage examined, a rather more pronounced degeneration in marrows far removed than in that into which the toxin was injected. As intravenous inoculation of like or larger amounts of toxin causes no more than a transitory marrow injury, the cause of the difference in effect is not clear.

4. It is suggested that the chronic experimental anemia, produced in this way may prove a useful means for evaluating the potency of extracts of liver and other organs as blood regenerating agents.

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DESCRIPTION OF PLATES

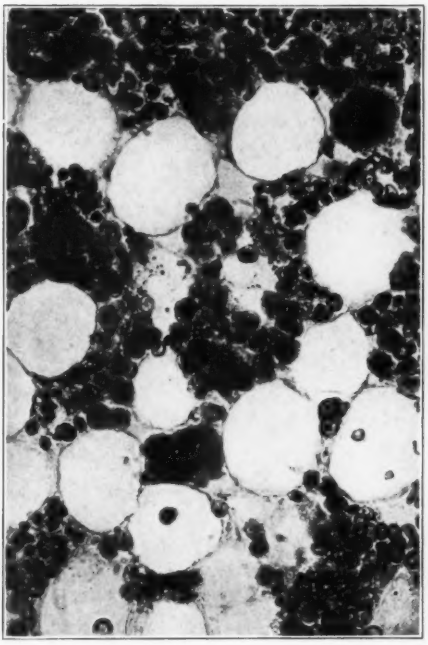
PLATE 29

FIG. 1. Normal rabbit, right tibial marrow. × 160.

FIG. 2. Rabbit 500. Right tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after eighteen hours. Atypical mitoses and degeneration of giant cells, many polymorphonuclear leucocytes. × 160.

FIG. 3. Rabbit 98. Right tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after twelve days. Extremely marked atrophy and degeneration of fat cells, numerous polymorphonuclear leucocytes. × 160.

FIG. 4. Rabbit 593. Right tibial marrow. Inoculated intravenously with 0.5 cc. *B. welchii* toxin. Killed after twelve days. Marrow rather cellular. Fat cells show mucous degeneration in moderate degree. Polymorphonuclear leucocytes very numerous. × 160.



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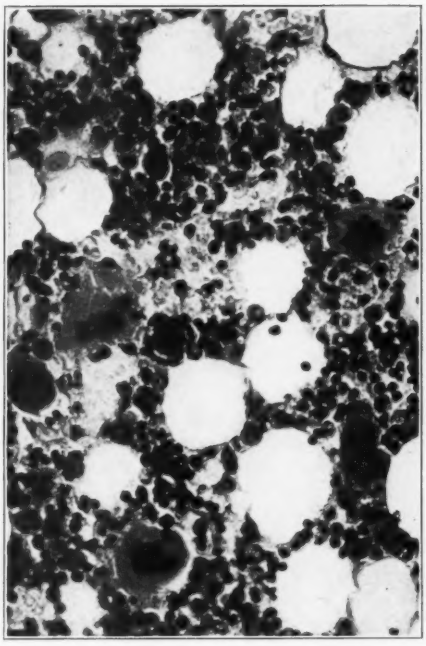

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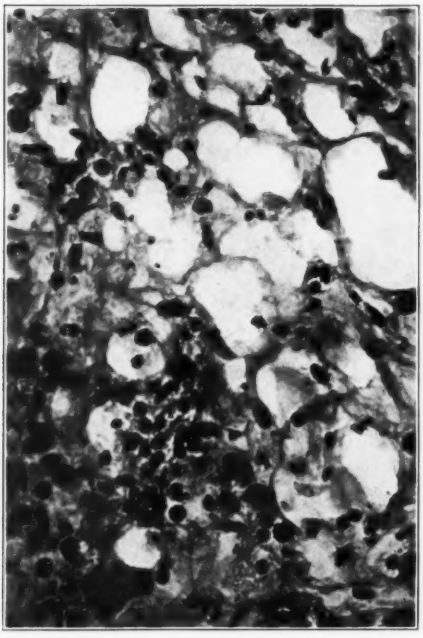

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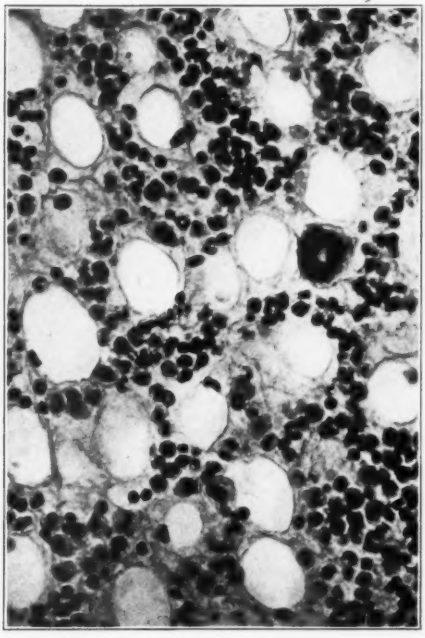


4

Torrey and Kahn

Progressive Anemia Following B. Welchii Toxins

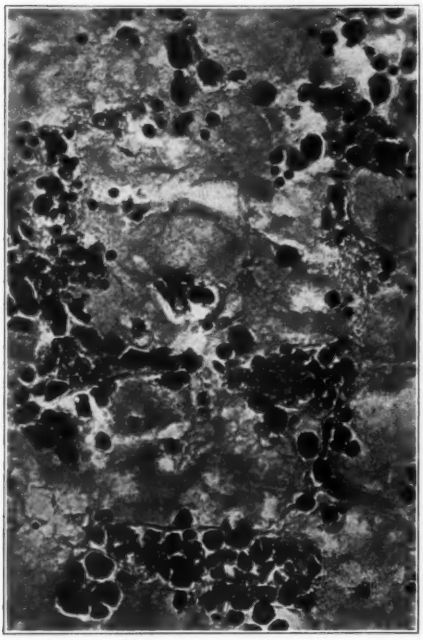
Plate 30

Fig. 5. Rabbit 254. Left tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Died seventy-five days later. Replacement of fat and lymphoid cells by a homogeneous granular material. The few cellular elements are mostly myelocytes and polymorphonuclear leucocytes.

Fig. 6. Rabbit 254. Right tibial marrow. Degenerative changes even more pronounced than for left tibia. Very few cells.

Fig. 7. Rabbit 579. Control for medium. Right tibial marrow. Inoculated into right tibial marrow with 0.5 cc. sterile broth medium. Killed after sixty-four days. No change except a simple atrophy with a marked reduction of all types of cells. Fat cells are normal.

Fig. 8. Monkey Q. Left tibial marrow. Inoculated into right tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after seventy-seven days. Marrow very cellular, comparatively few fat cells and very numerous lymphoid mononuclear cells.



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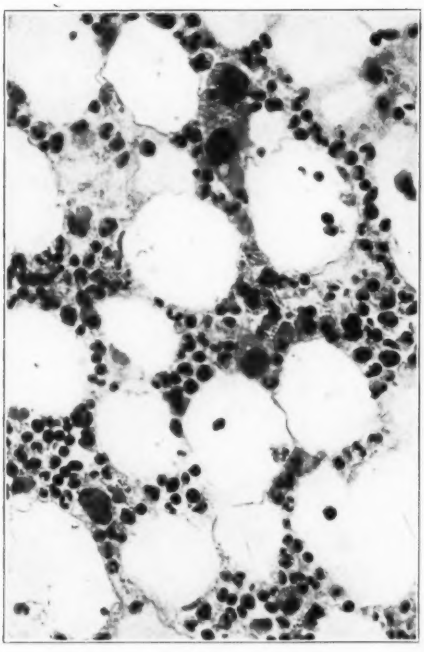

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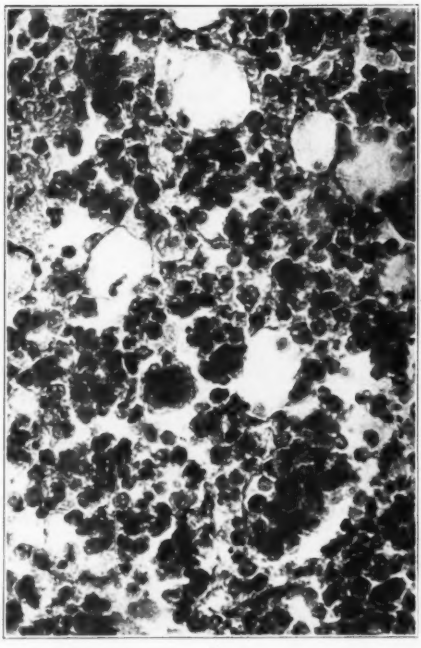

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Torrey and Kahn Progressive Anemia Following B. Welchii Toxins

HEMANGIOBLASTOMA OF THE CEREBELLUM (LINDAU'S DISEASE)*

LEO M. DAVIDOFF, M.D.

(From the Laboratory of Neuropathology, New York State Psychiatric Institute and Hospital, Ward's Island, N. Y.)

Beginning his study with two cases of cystic tumor of the cerebellum similar to the one about to be described, then searching the literature for other examples, Arvid Lindau,[1] the Swedish pathologist, first proved in 1926 the hemangiomatous nature of the tumors associated with these cysts, then showed the frequent association of these cystic tumors with angiomas of the retinae (von Hippel's disease) as well as frequent hemangiomatous and cystic tumors of the pancreas, kidneys, epididymi and other parts of the body.

That the combination of these various cysts and tumors in a single patient who frequently shows hereditary predisposition forms a definite syndrome, and may properly be known by the name of its discoverer, cannot be denied. The question may be asked, however, how many of the changes included in this syndrome must be present in a patient before his ailment may be properly classified as Lindau's disease.

In my case there was no hereditary taint, no angiomatosis of the retinae, no cysts or tumors of other organs. The cyst and tumor of the cerebellum, however, were identical with those described by Lindau. Since Lindau himself states that "the dominant and characteristic thing about this pathologico-anatomical complex is evidently the occurrence of angiomata in the central nervous system" and proposes the name "angiomatosis of the central nervous system" for it, I feel justified in considering my case one of Lindau's disease.

REPORT OF CASE

Clinical History: K. R. E., a German-American, 30 years of age, was admitted to the Bellevue Hospital, New York City, in December, 1905, complaining of headache, blindness and deafness. The headache began six months before. The loss of sight and hearing was of only one months duration.

Some unsteadiness was apparently noted but no definitely localizing symptoms. The family history was negative as far as could be determined.

* Received for publication January 2, 1929.

A month later a right parietotemporal decompression was done, and a dilated right lateral ventrical demonstrated. The operation resulted in temporary improvement.

Gradually the symptoms increased and were augmented by confusion, hallucinations and excitement.

June 22, 1906, he was transferred to the Central Islip N. Y. State Hospital, apparently severely demented; without sight or hearing, disoriented, muttering and incoherent. His optic discs showed a marked degree of choking. When stood upon his feet he fell to the left. As time went on, his head became retracted permanently, he became emaciated, and finally died August 26, 1907, of pulmonary infection.

Autopsy

A complete postmortem examination was made except for the spinal cord and retinae. Aside from pneumonia, nothing unusual was found in the neck, chest or abdomen.

Brain: The brain was large with a protrusion in the right cerebral hemisphere corresponding to the decompression. The lateral ventricles were markedly dilated.

The right cerebellar hemisphere was much larger than the left, displacing the pons and medulla toward the left. The pons was swollen and grooved by the basilar artery. The medulla was much compressed and the pyramids and olives flattened. The whole right cerebellar lobe was fluctuant and the folia more flattened than on the left. A small conical process of the tumor was apparent on the base of this lobe. A section through the protruding mass showed a tumor 4 cm. in diameter. The center was cystic and filled with viscid gelatinous fluid. The solid part of the tumor was reddish brown, hemorrhagic, of loose and spongy consistency. It seemed to be entirely encapsulated and could be easily shelled out (Fig. 1). Surrounding the growth was an area of tissue destruction, probably a result of pressure of the tumor. The cyst appeared to be entirely surrounded by tumor, even where the wall was much thinned out.

Grossly, the cut surface of the tumor showed the presence of small lacunae varying from a pin-point to a pin-head in size (Fig. 1).

Microscopic Examination: Low power examination shows the growth to be characterized by the presence of many smoothly lined lacunae of varying sizes with richly cellular tissue in between (Fig. 2). With slightly higher magnification the number of spaces proves to be much greater, and definite endothelium is seen lining them. Some of these spaces are filled with red blood corpuscles while others are entirely empty. Some of the larger spaces are actually cavernous

in character and possess, outside of the endothelium, a condensation of collagen fibers.

The endothelial cells of the larger vessels are flat with elongated nuclei as seen in ordinary blood vessels. In the smaller tumor capillaries the lining cells have rounder, larger, paler nuclei, which frequently protrude into the capillary luminae and tend to obliterate them.

The cells lying between the capillaries make up a considerable part of the tumor. They show no definite cell boundaries and have fairly large, oval, pale nuclei with evenly distributed scanty chromatin. The nuclei vary moderately in size and contain among them some that are several times larger than the average (small giant cells of Lindau).

With fat stains most of the cells show coarse discrete fat globules, which Lindau thinks makes them resemble xanthoma cells (Fig. 3).

Areas of homogenous, faintly staining colloid-like material are occasionally seen, representing in Lindau's opinion, evidence of insufficient blood supply (Fig. 4).

Connective tissue stains show collagen fibers in the walls of the larger sinuses and occasional fibers between the capillaries. No glia fibrillae are demonstrable within the tumor, and no astrocytes or spongioblasts are seen in preparations made with Cajal's gold sublimate impregnation.

Discussion

The identity of the tumor in this case with the hemangiomas of the cerebellum described by Lindau is obvious when compared with his summary of the characteristics of these tumors, which is:

1. Relatively small size.

2. Cortical or subcortical position in the lateral and posterior aspect of the cerebellum.

3. Sharp demarcation of the tumor from the brain substance.

4. Microscopic findings:

(*a*) Capillary hyperplastic hemangioma.
(*b*) Cavernous spaces.
(*c*) Pseudoxanthomatous cells.
(*d*) Small giant cell.
(*e*) Plasmatic transudate.

The occurrence of this type of tumor, though obviously rare, is proving, nevertheless, to be more common than one might think. In Lindau's monograph, for example, which was published in 1926, fifteen cases are cited, including Lindau's own as well as the cases from the literature. Within a year he was able to report upon four additional cases.[2]

Among Dr. Cushing's cases eleven examples of cerebellar hemangioblastoma are present. Three of these were autopsied and showed no complications outside of the cerebellum as was true of my case. Another of Dr. Cushing's cases which had been successfully operated upon was described in detail by him and Bailey.[3] This patient had an associated hemangioma of the retina, and also gave a history to the effect that his father and his father's sister had died of cystic tumors of the cerebellum.

As to the nature of these cystic hemangiomatous tumors, Lindau identifies them with the peritheliomas of Cushing and agrees with the latter "that certain of the rare tumors, like the peritheliomas, which arise from over the fourth ventricle may originate in the structures which Wislocki and Putnam have disclosed" in the *area postrema*. This is borne out by the fact that they occur in the brain only in relation to the rhomboid space, either in the posterior aspect of this space or in the lateral and posterior parts of the cerebellum. They never occur in the superior vermis where cerebellar gliomas have their favorite seat.

Summary

The practical importance of a knowledge of these tumors is in the first place that, where they occur alone, they are completely removable surgically with a resulting cure of the condition. Secondly, in the presence of all tumors of the cerebellar hemispheres, one must, knowing the associated lesions in cases of Lindau's disease, be on the lookout for *angiomatosis retinae* and tumors of the medulla, spinal cord, pancreas, kidneys, adrenals, epididymi, etc., especially if material gained by operation proves histologically to be hemangiomatous in character. The prognosis in the complicated cases should be very guarded even if the cerebellar tumor is removed in its entirety.

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DESCRIPTION OF PLATE

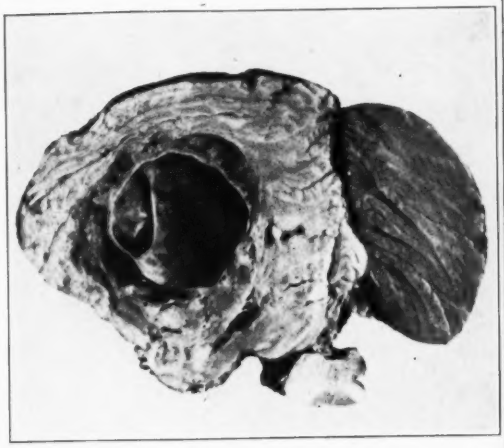
PLATE 31

FIG. 1. Actual size. Completely encapsulated cystic tumor of right lateral lobe of the cerebellum. The larger sinuses are easily discernible as small lacunae.

FIG. 2. The sharp demarcation of the tumor from the brain tissue may be seen, as well as the hemangiomatous character of the growth. H and E. × 80.

FIG. 3. Showing the discrete coarse fat granules in the tumor cells. Fettpenceau. × 290.

FIG. 4. An area of colloid-like coagulum seen here and there in these tumors. H and E. × 290.



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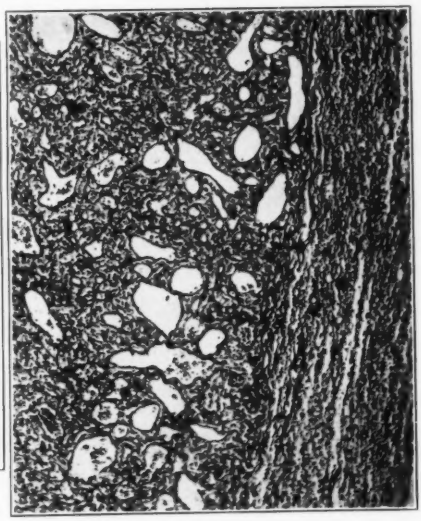

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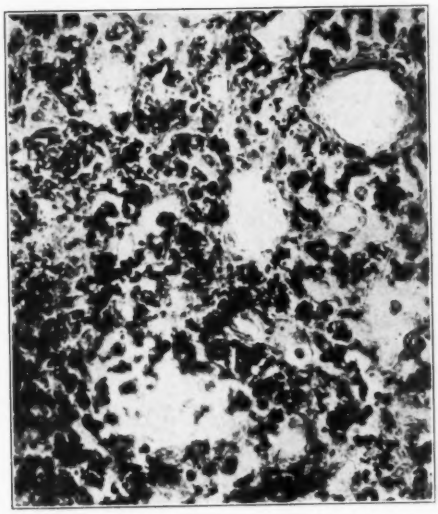

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Davidoff

Hemangioblastoma of Cerebellum

STUDIES ON FILTERABLE VIRUSES *

III. FURTHER OBSERVATIONS ON VACCINE VIRUS

ROBERT N. NYE, M.D., AND FREDERIC PARKER, JR., M.D.

(From the Thorndike Memorial Laboratory and the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

In two previous papers,[1,2] we have described certain experiments in which vaccine virus was successfully cultivated *in vitro*. It is our intention in this paper to report further studies on some of the cultural, biological and immunological aspects of this virus. Gordon [3] has recently published his exhaustive study of vaccine virus. In this, he has covered several points that we take up but since he was working with calf or rabbit lymph, while we used culture virus, we feel it worth while to report our results.

INCREASE OF VIRUS IN CULTURE

As described in one of the foregoing papers,[2] a strain of vaccine virus (VC 10) was cultivated for a period of fifty-six days, during which time it was transferred eleven times. Titrations of the virus content were made at various times and it was found that the eleventh generation contained 51,000 times as much virus as the original inoculum. We decided to repeat this experiment using the same methods for cultivation and titration.

The culture (VC 30) was started from bits of infected testis and normal plasma. The results are given in Table I. The actual virus content at the start was not determined, but after seven days incubation, it was equivalent to fifty skin doses per culture. The rate of increase on the whole was similar to that found in the previously described culture, VC 10. The twelfth generation of VC 30 contained a very large amount of virus. One-tenth of a cubic centimeter of a 1:50,000 dilution of two cultures gave a positive take. Since a culture weighed approximately ten milligrams or one-hundredth of a gram, a gram of this culture contained 25,000,000 skin doses. After the twelfth generation the potency rapidly diminished. The thirteenth contained only one-fifth as much virus, and the fifteenth less than one-hundredth as much virus as the twelfth gen-

* Received for publication November 21, 1928.

eration. Thereafter even the whole dilution failed to give a take. Such a record again demonstrates clearly that multiplication of the virus takes place under such conditions of cultivation.

The sudden drop in potency of VC 30 after about three months cultivation checks quite closely with another culture (VC 6A) with

TABLE I

Growth and Titration of Vaccine virus Culture, VC 30

Generation	Date	Split	Titration		Taking skin doses per culture	Virus content
			Amount used	Result		
	Aug.					
I	4		2 cultures	0.1 cc. 1:10+ 0.1 cc. 1:100−	50	1
II	11					
III	18					
IV	25		1 culture	0.1 cc. 1:500+ 0.1 cc. 1:1,000 not done	5,000	100
	Sept.					
V	1	yes				
VI	8		2 cultures	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000 ±	25,000	1,000
VII	15	yes				
VIII	19					
IX	26		2 cultures	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000−	25,000	2,000
	Oct.					
X	3					
XI	8	yes				
XII	15		2 cultures	0.1 cc. 1:50,000+ 0.1 cc. 1:100,000 not done	250,000	40,000
XIII	22	yes	1 culture	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000−	50,000	16,000
XIV	29					
	Nov.					
XV	5		1 culture	0.1 cc. 1:50− 0.1 cc. 1:10 not done	500	320

which positive skin takes were not obtained after ninety-seven days incubation. The reasons for such decreases in potency are at present obscure. They might be any one or all of these three factors: (1) diminution in virulence of the virus even although growth continues; (2) cessation of growth; (3) development of viricidal properties on the part of the tissues in the cultures. Further studies are necessary to elucidate this problem.

Certain Factors Involved in Virus Growth

(*A*) *Necessity of Living Tissue:* Having convinced ourselves that growth does take place beyond question, we then attempted to ascertain some of the factors involved.

Steinhardt and Lambert [4] concluded that living tissue was necessary for the growth of the virus. In certain of our control experiments in which the tissue was not washed or renewed and therefore died, we found that the virus did not survive. This was in accordance with the above-mentioned workers' views. In order to demonstrate this point more conclusively, cultures were set up using testis killed by freezing with a control of living testis. The cultures were set up with pieces of normal testis (one set frozen, the other untreated) and plasma to which had been added a known amount of culture virus. The control showed a strongly positive take in the first generation after incubation, whereas the culture made with killed testis was negative in both the first and second generations. Such a result confirms the view expressed above: namely, that living tissue is essential not alone for the growth but even for the survival of the virus. It also would argue against the view that the increase of virus in cultures is not due to multiplication but due to disintegration of inclusion bodies with the consequent setting free of the virus.

(*B*) *Anaerobiosis:* Plotz [5] in 1922 described the successful cultivation of vaccine virus in Smith-Noguchi tubes in which conditions closely approaching complete anaerobiosis must have been obtained. We therefore attempted to determine the effect of such conditions applied to our methods. As our culture dishes were sealed with vaseline and contained growing tissue, it seemed possible that the oxygen in the cultures might be exhausted and that the virus consequently was growing anaerobically. Therefore, the air in such a plate was analyzed for its oxygen and carbon dioxide content. It was found that oxygen was present and was but slightly lower than that of air under normal conditions; the carbon dioxide was slightly increased. Hence our cultures were not anaerobic. Next, to study this point further, we incubated cultures in an atmosphere devoid of oxygen. In no instance was there evidence of growth or even survival of the virus, for we were never able to get a positive take even with the whole dilution of the first generation. This apparent disagreement with Plotz' results made it seem advisable to attempt to repeat his

work. Using exactly his technic, we repeated his experiments with absolutely negative results. With an original inoculum many times as potent as his, we were unable to demonstrate any increase or survival of the virus. Since it seemed probable that testis would be a more favorable tissue than kidney, several series of tubes were set up using this tissue. A questionable take was obtained in one tube in the third generation but the duplicate tube was negative as were later generations. In short, all our attempts to repeat Plotz' work were unsuccessful.

Effect of Temperature

Using culture vaccine virus, we carried out some experiments planned to show the effect of different temperatures on the survival of the virus. In every instance the virus content in skin doses was known. The results are given in Table II. Twenty minutes at 55° C apparently completely destroyed the virus while shorter

Table II

Survival of Virus at Various Temperatures

Temperature	Duration	Result
55° C	5 min.	+++
	10 min.	++
	20 min.	–
37.5° C	24 hours	–
6° C	14 days	++++

In this and the succeeding tables, the +'s indicate the degree of intensity of the positive takes; a negative take is shown by the sign –.

periods showed decrease in potency. Gordon [3] found that thirty minutes at 55° C completely destroyed most samples of calf lymph although a few gave weak takes after such treatment. It is interesting to speculate whether such diminution is due to diminished virulence of all the virus or to whether an increasing proportion is killed and the takes are due to the few surviving. After twenty-four hours at 37.5° C no takes could be obtained while at 6° C the virus survived at least fourteen days. The suspensions of the virus in each instance were made in Locke's solution.

Influence of Hydrogen Ion Concentration

Culture virus suspensions of known strength were made in 50 per cent glycerine containing 5 per cent disodium phosphate, adjusted to the desired hydrogen ion concentration. The final dilutions were such that 0.1 cc. originally contained 1,000 skin doses. The tubes were kept at 6° C. The results are given in Table III. At pH 5.0, the

Table III

Survival of Virus at Various $[H]^+$ at 6° C

$[H]^+$	2 days	7 days	14 days	28 days
8.0	++++	++++	±	–
7.0	++++	++++	+++	–
6.0	++++	++++	++	–
5.0	++++	+++	–	–

virus content was diminished after seven days and no virus could be demonstrated after fourteen days. At pH 6.0 and pH 8.0, after seven days the virus was unchanged but at fourteen days was markedly weaker, especially in the pH 8.0 tube. At pH 7.0, the potency was undiminished after seven days and but slightly decreased after fourteen days. All tubes were negative after twenty-eight days. It would appear that the optimum pH for the survival of the virus in glycerine at icebox temperature is in the neighborhood of pH 7.0.

Effect of Centrifugalization

If vaccine virus is particulate, it is reasonable to expect to concentrate it by centrifuging. MacCallum and Oppenheimer [6] have described a differential method for such concentration. In our experiments a culture virus containing less than five skin doses per 0.1 cc. was centrifuged at about 2,800 revolutions per minute for one hour. The fluid at the top gave a moderately positive take when undiluted but diluted 1:10 was negative. The fluid at the bottom gave a strongly positive take and, diluted 1:10, a weak but definite take. Gordon [3] obtained similar results. In one of his experiments, the fluid before centrifuging was positive up to a 1:10,000 dilution, whereas after centrifuging, the top layer was positive only up to 1:1,000 while the botton layer gave a take at 1:50,000. Such evi-

dence would favor either the particulate nature of the virus or the association of the virus with particulate matter.

Filtration

Casagrandi [7] in 1909 was the first to filter vaccine virus successfully through a porcelain filter. Since then, both successful and unsuccessful results have been reported. Since we were working with a culture virus free from cells, we felt that conditions were favorable for filtration if such a thing was possible. Two experiments were carried out using a small Mandler filter. The tightness of the filter was controlled by adding to the virus suspension a loopful of bacteria from a twenty-hour throat culture on blood serum. Subcultures of the filtrate to plain broth were negative. The first virus suspension was such that 0.1 cc. contained 2,500 skin doses. The filtrate did not give a take. To rule out the possibility that the virus had passed through the filter but not in sufficient amounts to give a take, some of the filtrate was used to dilute normal plasma and cultures were set up with this and pieces of normal testis. The cultures were carried through three transfers and then tested with negative results. The second virus suspension was so diluted that 0.1 cc. contained five skin doses. All tests with the filtrate were negative. Gordon,[4] likewise, was unable to pass the virus through Berkefeld filters.

Viricidal Action of Hyperimmune Calf Serum

These experiments were made possible through the kindness of Dr. Benjamin White of the Massachusetts State Antitoxin and Vaccine Laboratory. A calf was hyperimmunized against vaccine virus by primary scarification followed by subcutaneous and then intravenous injections over a period of three months. Two weeks after the the last injection the calf was bled. Some of this serum was used by us in the following experiments. The normal serum employed was obtained from the same calf before immunization.

In the first set of experiments, cultures were set up in the usual manner except that instead of diluting the plasma with Locke's solution, it was diluted with two parts of either 50 per cent normal or 50 per cent immune calf serum. After seven days incubation each culture was ground with sand in the usual way and various dilutions were made and tested. The results are given in Table IV. The cul-

ture containing normal serum showed definite takes as high as 1:100 and questionable takes at 1:1,000 and 1:5,000 whereas that to which immune serum was added was negative at 1:10.

In order to titrate the viricidal action of the immune serum *in vitro*, varying dilutions of culture virus were incubated for one hour

TABLE IV

Viricidal Action of Hyperimmune Calf Serum in Cultures

Culture with Normal Serum		Culture with Immune Serum	
Dilutions	Results	Dilutions	Results
1:10	++++	1:10	−
1:100	++	1:100	−
1:1,000	±	1:1,000	−
1:5,000	±	1:5,000	−

TABLE V

Viricidal Action of Hyperimmune Calf Serum in Vitro
(Varying dilutions of virus + an equal amount of undiluted calf serum)

Virus Dilutions	Results with Normal Serum	Results with Immune Serum
1:1,000	−	−
1:100	+++	−
1:10	++	−

at 37.5° C with equal amounts of undiluted immune and normal serum. The various tubes were then tested as usual by intradermal injections. The results are shown in Table V. The virus was of such potency that 0.1 cc. of a 1:10 dilution contained 5,000 skin doses. The normal serum neutralized the 1:1,000 dilution but did not affect the 1:100 and 1:10 dilutions. The immune serum neutralized through the 1:10 dilution. Since 0.1 cc. of 1:1,000 virus contained $\frac{5000}{100}$ or 50 skin doses and since 0.1 cc. of normal serum neutralized this amount, then 1 cc. of normal serum could have neutralized 500 skin doses. Applying the same method of calculation, 1 cc. of the immune could have neutralized at least 50,000 skin doses.

Since the limit of viricidal or neutralizing power of the immune serum was not determined in the last experiment, another was set up using fixed amounts of virus, 0.1 cc. containing 500 skin doses, and

equal amounts of increasing dilutions of normal and immune serum. The tubes were incubated and tested as before (see Table VI). The normal serum failed to neutralize even when undiluted whereas the immune serum neutralized definitely at a 1:1,000 dilution. On the

TABLE VI

Viricidal Action of Hyperimmune Calf Serum in Vitro
(Varying dilutions of serum + an equal amount of virus, diluted 1: 10)

Serum Dilution	Results with Normal Serum	Results with Immune Serum
Undiluted	++	−
1: 10	++++	−
1: 100	+++	−
1: 1,000	++++	−
1: 10,000	++++	±

basis of this experiment, 1 cc. of normal serum could have neutralized less than 5,000 skin doses while the immune serum was capable of neutralizing as many as 5,000,000 skin doses. In the one experiment tried by Gordon, 1 cc. of immune serum neutralized 25,000 skin doses.

SUMMARY

1. Further experiments are described demonstrating the multiplication of vaccine virus in tissue cultures.

2. Vaccine virus could not be cultivated using killed tissues or under anaerobic conditions.

3. The virus when suspended in Locke's solution, was destroyed if heated to 55° C for twenty minutes or to 37.5° C for twenty-four hours.

4. The optimum hydrogen ion concentration for survival of the virus in glycerine was found to be pH 7.0.

5. After centrifugalization, the bottom layer contained more virus than the top layer.

6. The virus could not be filtered through a Mandler filter.

7. Experiments on the viricidal action of hyperimmune calf serum are described.

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NEUROEPITHELIOMA OF THE CEREBELLUM *

PAUL C. BUCY, M.D., AND WENDELL S. MUNCIE, M.D.

(From the Pathological Laboratories of the Henry Ford Hospital, Detroit, Mich.)

The authors, in reviewing the tumors of the central nervous system in the pathological collection of the Henry Ford Hospital, studied one tumor which is so rare in incidence and point of origin as to merit a place in medical literature.

According to Bailey and Cushing,[1] neuroepitheliomas are tumors composed of primitive spongioblasts characteristically arranged in rosettes about central canals, the canals lined by a cuticular membrane bearing blepharoplasten and presenting cilia upon its inner surface, and the outer extremities of the cells fading away as tails into the surrounding tissue. These authors state that the tumor commonly arises in the spinal cord and the retina. Flexner,[2] Wintersteiner[3] and others have described the retinal type, while Schlapp,[4] Rosenthal,[5] Thielen and Zinn,[6] and others have described the spinal cord type.

Bailey and Cushing in their series of 254 classified intracranial tumors had no examples of neuroepithelioma. However, in a subsequent article, Bailey[7] reported a case of neuroepithelioma of the left lower frontal lobe. The histological picture in this case corresponds very closely to the case reported here, as is shown by the photomicrographs and by sections of the tumor which we have been permitted to examine.

Just as we were about to submit this article for publication another case of neuroepithelioma was drawn to our attention, a very recent case of tumor involving the fourth ventricle. This case was operated upon by Dr. Harvey Cushing and as yet has not been fully studied. However, the sections which we have been fortunate enough to examine seemed typical of this pathological entity. It is also very interesting to note that the inner ends of the cells forming the rosettes bore definite cilia. This is undoubtedly due to the fact that the sections examined were from a specimen removed at operation and fixed immediately after its removal. Until this case is more

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completely studied, it cannot certainly be classed as a neuroepithelioma of the cerebellum.

In 1926 Naeslund[8] apparently described the first recognized case of neuroepithelioma of the cerebellum. This tumor occurred in a patient 18 years of age. It was found lying in the fourth ventricle and arose from the lateral margin of the anterior medullary velum. It had invaded and destroyed the ventral surface of the cerebellum. The photographs which accompanied the article and the description of the microscopic appearance are typical of neuroepithelioma. The columnar cells about the canals showed definite blepharoplasten in the inner ends with indistinct tails streaming into the surrounding tissue. No cilia were found.

From a perusal of the vast literature on the subject of brain tumors, two other observations were found of probable examples of true neuroepitheliomas: (1) a tumor arising near the aqueduct of Sylvius described by Roussy, Lhermitte, and Cornil,[9] and (2) a tumor of the left cerebral hemisphere described by Ribbert.[10] These two descriptions give meager detail of the finer cellular structure, but the drawings of the tumor seem characteristic of neuroepitheliomas. Possibly the tumor of the left hemisphere described by Uyematsu[11] might be a sixth example but it is difficult to judge from his brief description.

The above cases were the only examples of neuroepithelioma of the brain which a careful search of the literature revealed. "Neuroepitheliomas," it is true, are described in abundance, but a perusal of the descriptions convinces one that the term is used loosely to include ependymomas and ependymoblastomas, *i.e.*, any tumor arising from ependymal tissue and having any resemblance to epithelium. Under the title of neuroepithelioma gliomatosum, Roman[12] has described a tumor arising from the third ventricle very evidently composed of ependymal spongioblasts, and it would therefore be an example of ependymoblastoma, according to the classification of Bailey and Cushing. The tumor of the fourth ventricle described by E. Silberberg,[13] is doubtless another ependymoblastoma. This tumor showed pseudorosette formation, the lumina being blood sinuses instead of true central canals. The latter author refers to a tumor of the fourth ventricle described by M. Silberberg,[14] but there is no report of the histological detail. Cash[15] described a tumor of the lower medulla and upper cervical cord under the same title

which is unquestionably an ependymoblastoma, as the tails of the cells are well stained and arranged about blood spaces. Gordinier and Carey [16] described as a neuroepithelioma a tumor of the choroid plexus of the fourth ventricle which invaded the vermis. In the photographs it is seen that the rosettes have blood spaces for lumina. This is doubtless another ependymoblastoma.

Hirsch [17] under the title of neuroepithelioma, described a tumor of the fourth ventricle. There is no detailed histological report given, but according to the illustrations the tumor, although lying in the fourth ventricle, does not arise from the medulla or cerebellum, but extends up the dilated central canal from the cervical cord. We are unable to determine from the information given what type of tumor this is. Rosenthal [5] mentioned a gliomatous tumor of the fourth ventricle which was shown him by Glücksmann.[18] He describes this as containing passages lined with cylindrical epithelium, but furnishes no other detail. He also mentions a tumor of Ströbe's [19] which was found in the cerebral hemisphere and contained spaces lined with high cuboidal and cylindrical epithelium. Due to lack of detail in these cases it is impossible to classify them.

A tumor of the fourth ventricle arising from the posterior medullary velum is described by Muthmann and Sauerbeck.[20] This tumor is composed of columnar cells which occasionally line cavities and which possess well stained tails. These tails are gathered together in more or less thick bundles which radiate toward blood spaces. The authors are uncertain as to the presence of cilia on the inner surface of these cells. No mention is made of blepharoplasten. Bailey [21] has included this tumor in his description of ependymal tumors. This tumor bears a close resemblance to the one to be described here in that there seem to be true canals and the lining cells may have cilia. However, the authors are uncertain as to the presence of cilia, and canals are few. The tails stain unusually well, which is characteristic of ependymal spongioblasts, and we therefore would consider this tumor an ependymoblastoma.

The ependymomas have been described by Bailey.[21] Mallory [22] reported a case of ependymal tumor which was doubtless an ependymoma. Bassoe's [23] tumor of the fourth ventricle was said to resemble the tumor described by Muthmann and Sauerbeck, but according to the author's statement was less differentiated. From the description it would seem to be an ependymoma.

As to the remaining tumors which we investigated in the literature, it may be safely said that there is no example of neuroepithelioma fulfilling the criteria set down by Bailey and Cushing. This includes Greenfield's [24] series of forty tumors, Bassoe's [25] series of third and fourth ventricle tumors, and Tooth's [26] series of five hundred cases.

Neuroepithelioma of the brain is therefore very rare, four or possibly five having been described in contrast to numerous ependymomas and ependymoblastomas.

Report of Case

Clinical History: J. E. S., Case No. 57347, a boy of 5 years and 4 months, was admitted to the Pediatric Service of the Henry Ford Hospital on March 18, 1925, with the complaints of "stomach spells, dizziness, and pain in the back of the neck."

Family History: Negative.

Past History: Birth normal, spontaneous. Patient was said to have been cyanotic for two days after birth. Feeding normal. For the past two weeks the patient has had hiccoughs after eating, ending in vomiting. Development normal. Diseases, pertussis, varicella, rubella, possibly scarlet fever. Habits good. Weight 38 pounds, a loss of 4 pounds in the past two weeks.

Present Illness: Previous to the last three months the patient was quite well, playful, but whined more or less, the reason for which was unknown. He also cried easily.

At Christmas, 1924, he experienced a "stomach upset," began vomiting, especially in the morning after breakfast, and in the evening after supper, sometimes during the night. He always complained of nausea before the vomiting occurred. He has been constipated during this time, and has complained of abdominal pain on using enemas. Pain in the back of the neck had been sharp, and the patient held his head to one side (which side not stated). Recently he has had pain in both arms. During the last few days the patient has complained of double vision, and has kept the right eyelid closed. He staggers as if dizzy; seems tired all the time. He has lost four pounds in weight in the last two weeks.

Physical Examination, March 18, 1925: A well developed and well nourished boy. Weight 38 lbs., estimated normal weight 40 lbs. Temperature, 98, pulse 116, respiration, 24, blood pressure, systolic 75, diastolic 55.

General Examination: Coated tongue. Large cervical and axillary glands. Chest and abdomen negative.

Neurological Examination: Eyeslits normal; nystagmoid movements on fixation either to right or left, slight strabismus; pupillary reactions normal; bilateral papilloedema. All tendon reflexes normal; abdominals present. Gait, some staggering toward the left.

March 19, 1925: On this date there were noted: Macewan's sign negative; weakness of the left external rectus muscle, and diplopia; pupils, right slightly larger than the left, regular, reaction to light and on accommodation normal.

Lateral nystagmus, on fixation either to right or left, the quick component in the direction of fixation. Bilateral choked discs, two diopters of elevation. Tongue protruded to the right. Hearing impaired on the left, the patient hearing test-sounds at a distance of two feet on the left as compared with six feet on the right. Vestibular tests were said to indicate faulty conduction in the vestibular pathways bilaterally. Romberg positive, the patient falling to the left. Gait, a tendency to fall to the left was noted. Muscular twitchings of the arms and legs were observed during the night.

March 22, 1925: On this date a definite *tache cerebrale* was noted.

Laboratory Findings: Wassermann negative. Intracutaneous tuberculin tests negative to 1/20 and 1/10 mg. Blood and urine negative.

X-rays of the skull showed slight widening of the coronal suture.

Diagnosis: On admission a tentative diagnosis of encephalitis was made, with brain tumor and meningitis as other possibilities.

The final preoperative diagnosis lay between (1) tumor of the left cerebellopontine angle and (2) tumor of the left caudal portion of the pons. The patient was transferred to the Surgical Service for ventriculograms. Ventricular puncture was performed on March 23, 1925. On entering the right lateral ventricle the cerebrospinal fluid spurted out, evidently under increased pressure, but this was not measured. X-rays taken after injection of air into the lateral ventricle showed both lateral ventricles and the third ventricle to be enlarged.

Ventricular Fluid: Pandy negative, cells 1, Kolmer negative, Lange and mastic curves negative throughout.

The patient returned from the operating room in good condition and remained so until about 5.30 A.M. on March 25, 1925, when his condition suddenly became very poor, and he died at 6.00 A.M. of the same day.

Permission was obtained for postmortem examination of the brain only.

Necropsy (No. 319) was performed at 2.30 P.M. on March 25, 1925, by Dr. C. Z. Garber. The verbatim report follows.

Necropsy Protocol

External Appearance: "The body is that of a well nourished, well developed little white boy. Embalming has been done and permission is for examination of the brain only. In the right parietal region the hair is shaved, and there is a 3 cm. long V-shaped recent surgical incision wound. It is located about 3 cm. from the midline and 7 cm. from the external occipital protuberance. Below the wound there is a small round hole in the skull.

Brain: The meninges are not thickened. The dura below the trephination wound shows a 2 mm. hole, the borders of which are slightly roughened by small blood clots. Venous sinuses are open. The brain weighs 1330 gm. Convolutions are slightly flattened, and the sulci are rather shallow. Occupying the inferior surface of

the left cerebellar lobe and extending over the pons and medulla is a whitish gray lobulated tumor mass, a little firmer in consistency than the brain itself. The pons is pushed to the right and is very much compressed on the left side by an overgrowth of tumor. The edges of the tumor are free so that the mass can be shelled out to some extent. An attempt is not made, however, to shell out the whole mass. Lobules of the tumor extend posteriorly over the vermis and down over the posterior surface of the medulla and cord. Some of the lobules actually project into the foramen magnum. The tumor measures 6 cm. laterally, 7.5 cm. anteroposteriorly. It appears to be about 2.5 cm. thick. Several brownish red areas are visible on the tumor surface, and near the right edge there is a purplish soft area. The left trigeminal nerve is very much elongated and passes around the tumor. The left abducens nerve is a little longer than the right. The left facial and acoustic nerves emerge from the tumor mass, and are entirely surrounded by it. The edge of the tumor just touches the left glossopharyngeal, vagus, and hypoglossal nerves. The basilar and left vertebral arteries are pushed to the right by the tumor, and the left vertebral seems to have been somewhat compressed. Lobules of the tumor press on the orifices of the foramen of Magendie and the left foramen of Luschka. The right foramen of Luschka seems to be partially occluded, due to transmitted pressure of the medulla against the right cerebellar hemisphere. In the right parietal region beneath the trephination wound, there is a small puncture wound in the cortex. On section, the third ventricle and the lateral ventricles are somewhat dilated. At the level of the aqueduct there is a small puncture wound in the roof of the fourth ventricle near its medial border. The pons is found to be very much compressed and pushed to the right. The fourth ventricle is distorted and in one place is not much more than a narrow slit. At this level the tumor is not actually attached to the brain. It has a fine spongy grayish white appearance, and toward the center of the mass it shows a coarse, spongy, reddish yellow area dotted with flecks of old hemorrhage. At the level of the dentate nuclei and the inferior olivary nuclei the tumor is firmly attached to and replaces the inferior part of the left cerebellar hemisphere. Its progress upward is limited by the smooth edge of the white matter, but in places it merges imperceptibly with the gray matter. There are several cysts filled with translucent gelatinous, greenish gray

material, the largest being about 1 cm. in diameter. The best preserved part of the tumor has a slightly spongy, opaque, grayish white appearance. Small hemorrhages and bright yellow flecks present a striking contrast (Fig. 1).

Provisional Anatomical Diagnosis: Glioma of cerebellum. Pressure occlusion of the fourth ventricle. Slight hydrocephalus."

A section of the tumor was stained with hematoxylin and eosin, and the histological diagnosis of glioma was made.

Microscopic Examination

Technique: Material was refixed in formalin nine hours postmortem and embedded in paraffin. Sections were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, neutral ethyl violet-orange G, Van Gieson's acid picro-fuchsin, del Rio-Hortega's first and fourth variants and Weigert's iron hematoxylin. Sections were also impregnated according to Cajal's method for non-medullated nerve fibers but carried to such a degree that the method lost its specificity. The results although serviceable were far from perfect, due to previous embalming.

Microscopic Examination: The characteristic and predominating cells in this tumor are columnar, arranged for the most part about small canals or cavities forming true rosettes (Figs. 4 and 5). The major portion of the cytoplasm, *i.e.*, the columnar portion, lies between the nucleus and the canal, while the peripheral end of the cell, that portion just beyond the nucleus, is indefinite, and fades away into the surrounding tissue. One can make out vague and indistinct fibrous processes, which arise from the cell at this point and stream out into the "interstitial" tissue. These processes are not stained by the usual procedures, although in a very few instances in the sections prepared by del Rio-Hortega's fourth variant a very few definite processes can be detected.

By using the silver pyridine technique described by Cajal for impregnating non-medullated nerve fibers, but carrying the method to a point where it lost its specificity, the tails of these cells were beautifully impregnated in one only of a series of sections (Fig. 6). This procedure was first used by Bailey in his attempt to demonstrate the cells in the case he reported. In applying the method to this case we found that the body of the cell rapidly tapered off into

a narrow filament which frequently terminated on a blood vessel and in other cases ended in a bulbous enlargement.

In all stains, but particularly in those sections stained by Mallory's phosphotungstic acid hematoxylin, the irregularity of the outer ends of the cells, and the complete absence of any external limiting membrane is emphasized (Fig. 5). The cytoplasm of these cells consists of a finely granular eosinophilic material. At the very innermost border of the cell lies a thin cuticular membrane bearing numerous darkly stained minute rod-like and spherical bodies. These are especially well seen in the phosphotungstic acid hematoxylin and neutral ethyl violet-orange G preparations. They are undoubtedly blepharoplasten (Fig. 3). (Compare with the illustration in Bailey's Atlas.)[27] On this same surface, lining the canals, can be seen in a few instances minute processes which seem to be cilia. They are indistinct, however, and may be degenerative products or other débris.

The nuclei are small and of uniform size, about 10 microns in diameter. For the most part they are spherical with a moderate amount of finely granular chromatin scattered throughout the nucleus, slightly more at the periphery than elsewhere. In a few instances one large darkly staining granule is present, but most of the nuclei have no nucleoli. A few mitotic figures are seen, but they are far from numerous.

The rosettes described above, although found throughout the tumor, are most numerous at the periphery where they predominate almost to the exclusion of all other forms of cellular organization. Toward the interior and base of the tumor numerous variations are seen. There are many cells grouped in rosette formation without a central lumen, the cells uniting with those of the opposite side. On the other hand, there are many instances of large cavities lined by these same columnar cells. The walls of some of these large cavities are thrown into many folds (Fig. 2). Occasionally a large cavity filled with red blood cells is seen. This is evidently the result of hemorrhage into the cavity, for the wall of the cavity is composed of columnar epithelium.

In the space between the rosettes and the cavities are many cells identical with the columnar cells but in which the cytoplasm has become indistinct in outline. These cells are massed together with no attempt at organization (Fig. 7).

The surface of the tumor in many places is covered by a single layer of cuboidal cells indistinguishable from ependymal cells.

Some sections of the tumor include neighboring areas of apparently normal cerebellar tissue. At the point of junction of the neoplastic tissue with the cerebellum the comparatively lightly stained nuclei of the tumor intermingle with the somewhat smaller and much darker nuclei of the granular layer of the cerebellum. This intermingling of the two tissues is such that there is no definite line of separation between them (Fig. 7). In and near these areas the tumor cells are in unorganized masses. In a few places along this line of junction marked proliferation of the capillary endothelium has occurred.

The tumor is quite vascular, containing many large and thin-walled vessels, well visualized with Van Gieson's acid picro-fuchsin stain.

The areas about the blood vessels for a short distance are entirely free of cells, and are filled with fibrillary material (Fig. 6). This is especially evident in the section impregnated by Cajal's method and less so in those by Hortega's fourth variant. In the latter the cells arranged about the smaller vessels present definite, though poorly impregnated, fine fibrous processes which radiate toward the vessel walls. This is typical of primitive spongioblasts, according to Penfield.[28]

The sections stained by the phosphotungstic acid hematoxylin method, and those by Hortega's first variant even more clearly, show areas of marked formation of glia fibers. These are formed into an intricate network which is particularly dense at some points near the periphery of the tumor. In the less dense areas of this tissue a few bipolar cells can be found in the silver-stained sections.

Throughout the tumor, but particularly in that portion farthest from its base, are many areas of degeneration. These are definitely necrotic and many show hemorrhagic infiltration. The center of the degenerated masses, some of which are large, even 1 to 2 cm. in diameter, are acellular, and at the periphery a few poorly stained nuclei are seen (Fig. 8). These degenerated areas can also be clearly seen in the gross specimen (Fig. 1).

In one section the relation of the tumor to the ependymal lining of the fourth ventricle and to the medulla are well shown. The orderly arrangement of the cuboidal ependymal cells rather sud-

denly gives way to the tumor mass. The growth for the most part is outside the ventricle. The medulla is compressed but intact.

Schlapp states that there is a central gliosis in almost all cases of neuroepithelioma of the cord. Sections from the upper cervical cord and from the fourth ventricle in this case showed entirely normal ependyma with no evidence of cellular proliferation. The glia fibers surrounding the central canal are slightly increased in number and the walls of the central canal show some slight tendency to infolding. Sections of lower portions of the cord were not obtained.

Discussion

The primitive spongioblast has been described by Bailey and Cushing and by Penfield as a direct development of the medullary epithelium of the neural tube. These spongioblasts are cells with oval nuclei, whose inner ends form the ventricular surface of the developing nervous system, while the outer extremities continue off as long tails to the external limiting membrane of the neural tube, and also, as Penfield has pointed out, to neighboring blood vessels. His [29] and Castro [30] have shown that these cells present cilia on their inner surfaces and blepharoplasten at the base of the cilia. That these cells are the cells forming the major part of this tumor there can be no doubt.

Primitive spongioblasts have been shown to be the forerunner of bipolar spongioblasts by Castro, and this fact readily lends itself to the explanation of such bipolar cells in this tumor.

The roof of the fourth ventricle has long been recognized as the site of origin of a large proportion of cerebellar tumors. Bailey and Cushing have pointed out that the indifferent cells (medulloblasts) may occasionally be found near the ependymal lining of the ventricles, and cerebellar tumors composed of such cells and arising from the roof of the fourth ventricle are very common. Twenty-five of their twenty-nine cases of medulloblastomas had this origin. Pfleger [31] has also pointed this out as a common location for heterotopic groups of cells.

It is well known that in the adult nervous system the roof and floor plate are points of arrest in the development of ependymal cells. Streeter [32] states: "In the region of the anterior median fissure of the cord . . . the neuroglia maintains its primitive ependymal

type of simple radial fibers extending from the lumen to the surface of the cord." He also points out that such primitive spongioblasts can be found in the posterior median septum of the cord. So in view of the prevalence of embryonic rests of this type of cell in the cord it is to be expected that neuroepitheliomas of the cord would be common.

Referring to the medulla and cerebellum, Streeter says: "The fourth ventricle is completely covered by the expanded roof plate consisting of a thinned-out layer of ependymal cells which is attached laterally to the border of the alar plate, the transitional line forming the rhombic lip." And continuing: "At the rhombic lip are found cells which retain their primitive embryonic appearance into adult life," and "the ependymal cells at the rhombic lip differ from the rest of the ependyma in that they continue to show active proliferation late into embryonic life. The same feature is shown throughout the whole rhombic lip but is more marked in the portion belonging to the cerebellum. . . . It is apparent that the cortex (cerebellar) formation begins at the rhombic lip."

Thus it would seem that this area would be one of the most probable for the development of neuroepitheliomas, and it seems most likely that the tumor described, originating from the lateral wall of the fourth ventricle and invading the cerebellum, arose from the rhombic lip, the resting place of primitive spongioblasts in adult tissues, and the point of origin of the cerebellum.

Considering the prevalence in this region of tumors composed of other types of embryonic cells, medulloblastomas, ependymoblastomas, and ependymomas and the existence of an area of primitive spongioblasts at this point, it is to be wondered at that neuroepitheliomas of the cerebellum, such as the present case, are not more common.

Summary

A case of intracranial tumor in a white boy, aged five years and four months, duration of three months from the first definite symptoms to sudden death following ventricular puncture, is presented. The tumor is situated in the left inferior cerebellar lobe, displacing the fourth ventricle, and overhanging the medulla and cord. It is 6 by 7.5 by 2.5 cm. in dimensions, lobulated, vascular, cystic, and with hemorrhagic infiltration in the degenerated areas. Histologi-

cally the tumor is a true neuroepithelioma, according to the criteria set down by Bailey and Cushing for this type of tumor. As such it belongs with the brain tumors described by Naeslund, Ribbert, Roussy, Lhermitte, and Cornil, and by Bailey. It is the second neuroepithelioma situated in the cerebellum to be described.

Conclusions

1. Neuroepitheliomas are composed of primitive spongioblasts, and, as such, arise from the parent cells of ependymal tissue.

2. Though more commonly arising from the ependyma of the spinal cord and from the retina, they may arise from ependyma elsewhere.

3. A review of the literature shows that the case here reported is the fifth authentic case of neuroepithelioma of the brain, and the second involving the cerebellum.

Note: We wish to thank Dr. Frank W. Hartman for having placed the pathological material and the facilities of his laboratories at our disposal, for the photomicrographs which he made, and for his kindly assistance and interest.

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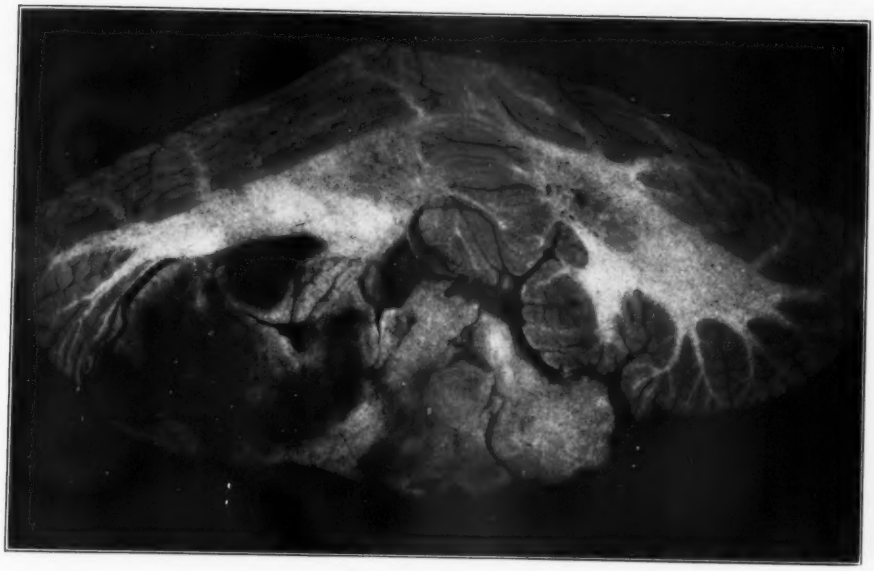
DESCRIPTION OF PLATES

PLATE 32

FIG. 1. Section through the tumor at its center showing its extent, invasion of the cerebellum, and relation to the fourth ventricle and the compression of the pons. The numerous areas of degeneration and hemorrhagic infiltration can also be seen.

FIG. 2. Another portion of the tumor showing large cavities lined by primitive spongioblasts. The walls of the cavities are thrown into folds. The cavity on the right contains many erythrocytes, but is obviously not a blood vessel. Many rosettes can be seen in the intervening tissue. Hematoxylin and eosin.



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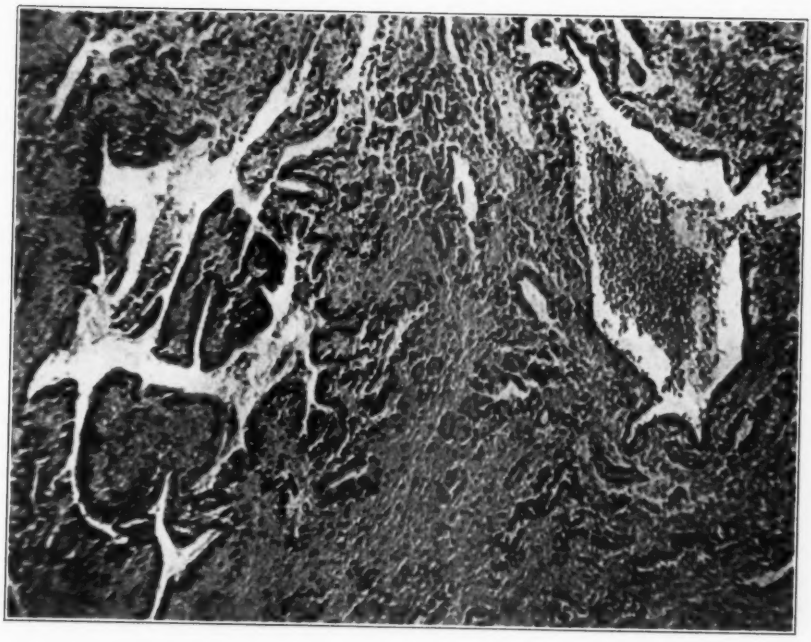


2

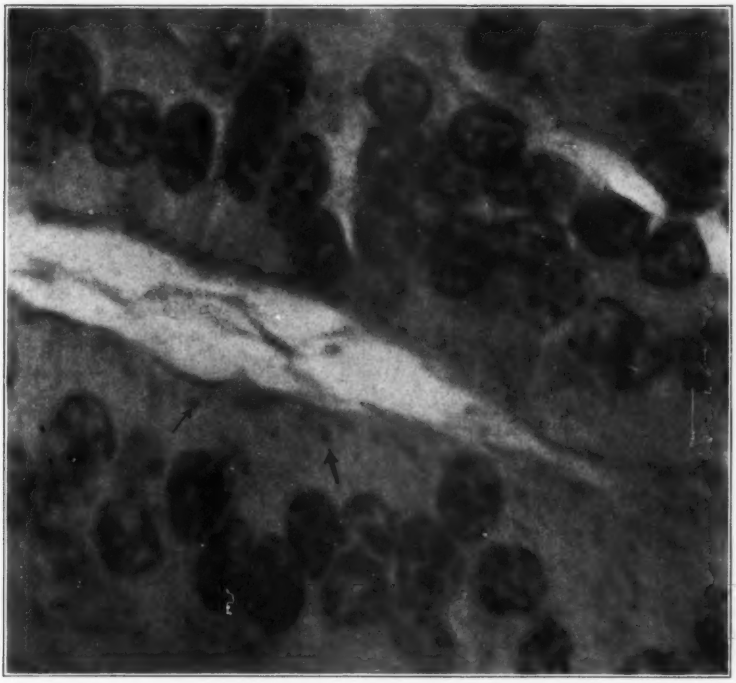
Bucy and Muncie Neuroepithelioma of Cerebellum

PLATE 33

FIG. 3. Single rosette showing blepharoplasten at the inner ends of the cells. Mallory's phosphotungstic acid hematoxylin. Very high magnification.

FIG. 4. Characteristic appearance of the tumor at its periphery, showing numerous rosettes. Hematoxylin and eosin. Low magnification.

FIG. 5. Rosettes at higher magnification than in Fig. 4. The absence of a limiting membrane, and the cuticular lining of the canals are characteristic features. Note that the peripheral extensions of the high columnar cells forming the rosettes do not stain. Mallory's phosphotungstic acid hematoxylin.



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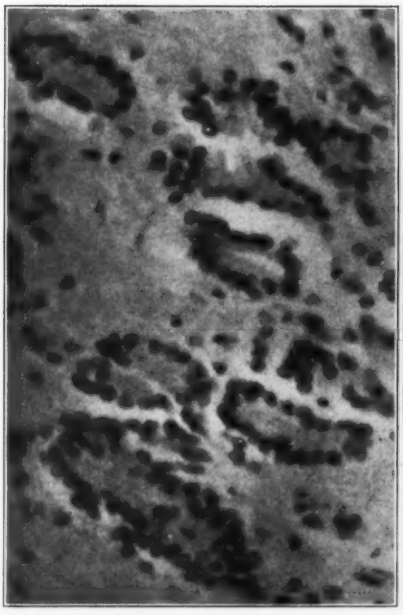

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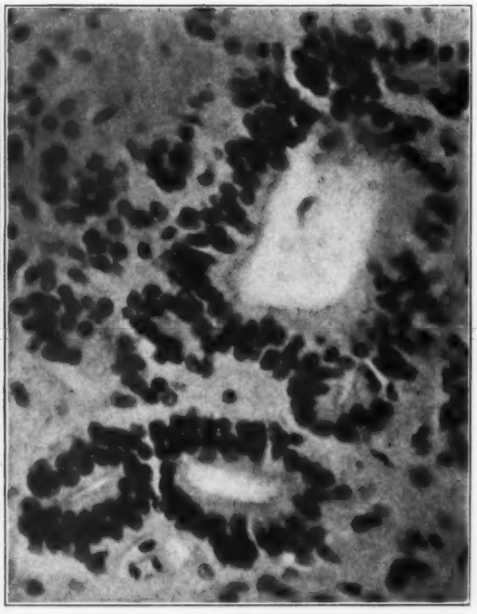

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Bucy and Muncie

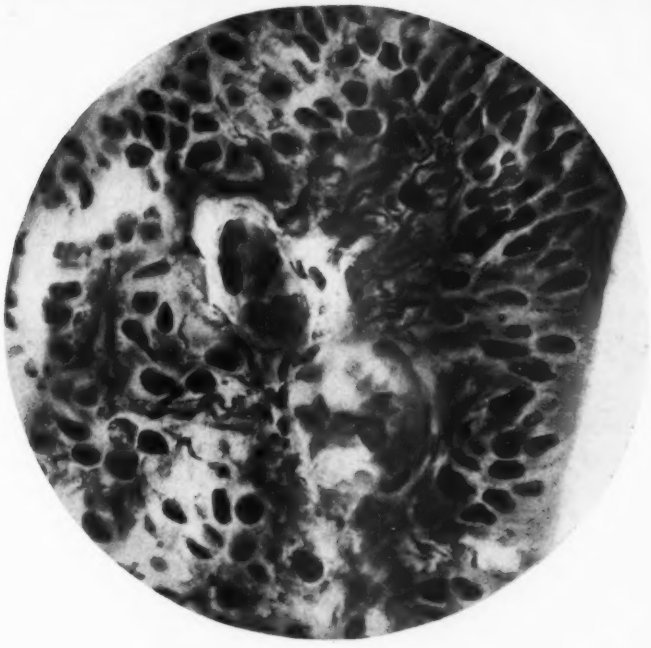
Neuroepithelioma of Cerebellum

PLATE 34

FIG. 6. Photomicrograph taken at the edge of a rosette, the edge of the canal lies at the right margin, with blood vessels in the center of the field. The tails of the cells which are shown very clearly can be seen running from the cells about the canal to the neighboring blood vessels. Cajal technic for non-medullated nerve fibers, overstained, non-specific. × 800.

FIG. 7. Junction of the cerebellum and the tumor. The intermingling of the lightly staining indifferent cells of the tumor below with the darkly staining smaller cells of the granular layer of the cerebellum above is well shown. The tumor cells at this point show no tendency toward organization. Their cytoplasm is very indefinite. Hematoxylin and eosin.

FIG. 8. Margin of an area of massive degeneration from the center of the tumor. A few cystic cavities can be seen on the left. Hematoxylin and eosin.



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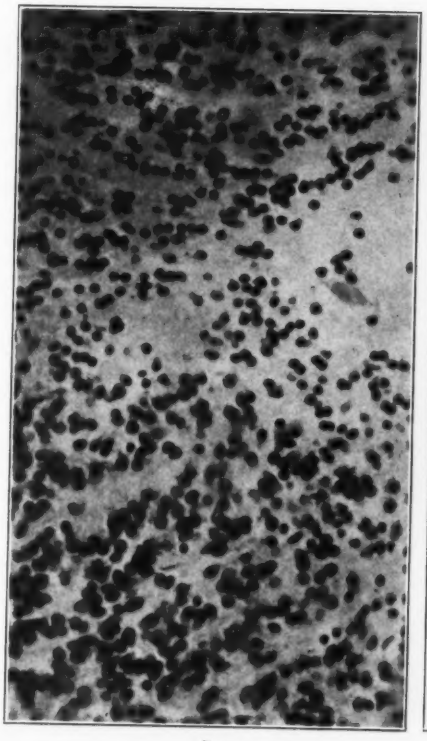

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Bucy and Muncie

8

Neuroepithelioma of Cerebellum

THE EFFECT OF COMBINED FEEDING OF POTASSIUM IODIDE AND ANTERIOR LOBE OF THE PITUITARY UPON THE THYROID GLAND *

HOWARD ANDERSON MCCORDOCK

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

In a series of studies on compensatory hypertrophy of the thyroid gland Loeb [1] has shown that the oral administration of potassium iodide in partially thyroidectomized animals not only fails to prevent hypertrophy but may even stimulate regenerative activity, while the feeding of anterior lobe of pituitary gland has an inhibiting effect preventing even moderate degrees of hypertrophy and causes a hardening of the colloid and a lowering of the acinus cells. The effect of potassium iodide on the normal intact thyroid has also recently been studied and a method evolved for determining the proliferative activity in a definite and quantitative manner by estimating the number of mitoses in the gland at any given time.[2, 3] By this means the stimulating effect of iodine on mitotic proliferation in the thyroid has been established.

In view of the antagonistic action of these two substances on thyroid activity in compensatory hypertrophy, it seemed interesting to see if the stimulating effect of potassium iodide would counteract the inhibiting action of anterior pituitary substance, or vice versa, when both are fed to the same animal. If these two substances completely neutralized each other, as far as their influence on the thyroid is concerned, it should remain unaltered, but if one were dominant the degree of its influence could be ascertained by estimating the mitotic activity of the gland.

The experiments to be described concern the effect of such combined feeding with potassium iodide and anterior pituitary on the thyroid gland; the result of administering each of these two substances separately to different animals and a comparison of these three types with normal thyroids from control guinea pigs.

Three series of experiments were carried out. Guinea pigs were used and in each series there were four groups: (*a*) animals fed with

* Received for publication October 26, 1928.

KI, each receiving a daily dose of 0.05 grams, made up in a pill; (*b*) those fed one 5 grain tablet of anterior pituitary (Armour & Co.) every day; (*c*) others receiving daily both the 5 grain pituitary tablet and the 0.05 gram pill of KI; and (*d*) control animals. The four groups of each series were kept under as nearly identical conditions as possible. All received the same kind of food and at the end of the experiment were killed with chloroform and the thyroid glands were fixed in Zenker's fluid. Serial sections were made of all the thyroids.

The number of mitoses in each gland was computed by counting those in every tenth section. This estimate constitutes a reliable index of mitotic proliferation in the thyroid gland. In studying the sections the size of the acini, the character of the cells and the conditions of the colloid were compared.

I. The Number of Mitoses

Animals Fed with KI: In the first series five guinea pigs were fed KI for twenty days and showed an average of 498 mitoses in the whole thyroid. The number of cells in division in this group was less than had been encountered in previous experiments in this laboratory. This difference could be partly accounted for by a slight loss in weight sustained by some of the animals during the course of the experiment, for it has been observed (by Loeb and by Rabinovitch) that the degree of compensatory hypertrophy diminishes and that potassium iodide fails to call forth the maximum karyokinetic response during periods of underfeeding when the animals are losing weight. However, some of these guinea pigs gained in weight and still showed lower counts than one would expect from the histological character of the sections, suggesting that perhaps this batch of animals happened to be more sensitive to KI and that therefore at twenty days the period of maximum activity had already passed and the thyroid was then approaching the next stage in which mitotic activity shows a noticeable decline. Because of this experience, in the second and third series the animals were fed KI for a period of only fifteen days. The average number of mitoses for both these groups was 1919.

Pituitary-Fed Animals: Altogether ten animals were fed each a 5 grain tablet of anterior pituitary daily. In five, the feeding extended over a period of twenty days and in five, for a period of

TABLE I

First Series

Potassium Iodide Feeding		Pituitary Feeding	
Guinea pig	No. of Mitoses	Guinea pig	No. of Mitoses
1	370	11	35
2	568	12	56
3	392	13	21
4	460	14	48
5	700	15	70
Average	498	Average	46

Second Series

6	1710	16	74
7	1697	17	55

Third Series

8	2130	18	80
9	2250	19	107
10	1810	20	76
Average	1919	Average	78
Average for all three series	1208.	Average for all three series	62.

First Series

Potassium Iodide and Pituitary Feeding		Controls	
21	Died	33	85
22	"	34	130
23	"	35	92
24	150	36	196
25	214		
Average	182	Average	125

Second Series

26	142	37	170
27	160	38	60
28	121	39	40
29	80		
Average	125	Average	90

Third Series

30	96
31	130
32	110
Average	112
Average for all three series	133
Average for all controls	110

fifteen days. The average number of mitoses was 62 for these ten animals.

Combined Feeding with KI and Anterior Pituitary: The average number of mitoses in this group, comprising nine animals, was 133. In addition there were three animals in the first series which died before the twentieth day and were not included in this list.

Control Animals: In the control animals the average number of mitoses was 110 per gland. A summary of the mitotic counts will be found in Table I.

II. Effects of KI and Pituitary Separately and in Combination on the Structure of the Thyroid Gland

Control Animals: The normal guinea pig thyroid is composed of small to medium-sized acini, the largest ones usually being situated at the center of the gland. Three zones of acini can usually be distinguished; the peripheral and central areas contain large acini and the intervening zone is made up of smaller acini. Low cuboidal epithelial cells with prominent nuclei line the acini that contain solid, homogeneus, intensely staining, slightly retracted colloid. There is, however, considerable variation in structure in different animals. The size of the acini may vary and at times the colloid is somewhat softer, staining less intensely with eosin and showing vacuoles in the peripheral zone. Phagocytic cells are now and then seen in the colloid, though this is a rare occurrence in normal glands. In older guinea pigs collections of lymphocytes may occasionally be found in the connective tissue of the stroma.

Potassium Iodide-Fed Animals: The histological appearance of thyroids from animals fed with KI has been dealt with at length in papers by Gray [2] and Loeb and Rabinovitch.[3] The changes in the thyroids of this group are essentially the same as those described by them. Briefly, the changes in the first series that were fed for twenty days are as follows: The acini are larger than in the controls and, at first glance, their epithelium seems on the whole more flattened than normally, except where there is mitotic division. This apparent flattening, however, is evidently due to the pressure exerted on the epithelium by the increased content of the acini and one can readily see that in places where no pressure is exerted on the acinar cells they are larger than normally, and even in large distended acini

where they are spread out to cover a larger surface their volume is unquestionably greater than that of the same number of cells in control animals. A feature concomitant with these changes is a great reduction in the amount of solid colloid, it being replaced by liquefied colloid which either stains very lightly or does not stain at all. Under low power the great variation in the amount of solid and liquefied colloid in the different acini is very striking. Groups of phagocytic cells are found in the colloid within many of the acini and form a very marked feature in this twenty-day series.

The changes observed in the two fifteen-day series are not as obvious as those just described. The size of the acini and character of the colloid do not differ much from the controls. The epithelial cells, however, are somewhat hypertrophied and increased in number. They also show many mitotic figures. The number of phagocytes in the colloid is not as great as in the twenty-day series.

Anterior Pituitary-Fed Animals: Thyroids of this group show acini crowded closely together and completely filled with a very solid intensely staining and non-vacuolar colloid. The absence of retraction of the colloid from the lining cells is a distinctive feature of the pituitary-fed animals. Everywhere the epithelium is very low as if compressed by the colloid in the distended acini. At the center of the gland an occasional large acinus has a slightly softened, pale, bluish colloid, and it is in the epithelium of these that most of the remaining mitotic figures are found. The acini in this group are also on the whole noticeably smaller than in the controls.

Combined Feeding with KI and Anterior Pituitary: Thyroids from these animals show certain variations depending upon the length of feeding. Those fed for twenty days (only two animals) have a central zone composed of a small number of rather large acini some of which contain a soft, light-staining colloid. In some of these acini a few phagocytic cells are found, though they are not numerous. The rest of the thyroid about this central zone is composed of small to medium-sized acini distended with a dense solid colloid extending to the cells but not compressing them as in the pituitary animals. In this outer zone the acini are close together but are not often crowded sufficiently to obscure all traces of the loose connective tissue stroma seen in normal glands.

Those animals fed for only fifteen days show essentially the same changes except that the central zone of large acini with softened

colloid is absent and acini with solid colloid are seen throughout the gland. Even in this series an occasional acinus with pale-staining colloid is found in the center of the thyroid but phagocytic cells are entirely lacking. The acini are small and are lined by a low to medium-sized epithelium but are not as noticeably compressed or flattened as in the twenty-day series. The acini are in some places close together but for the most part are slightly separated by thin strands of connective tissue.

The appearance of these thyroids stands midway between those from normal and those from pituitary-fed animals. Their acini are about the same size as in the normal gland but are separated by less stroma. They are, however, not as crowded as in the pituitary animals. The epithelium is slightly flattened but not compressed into a thin strand of cells as is often seen in the pituitary group. The colloid is hard, stains intensely with eosin and completely fills the acini; it is not vacuolar and is uniform in amount, thus differing from the normal.

These glands then show the influence of anterior pituitary substance. It might be possible to so balance the dosage of these two substances that the thyroids would retain their normal characteristics and not approximate the condition found in animals fed either with KI or with anterior pituitary gland.

Discussion

From these experiments it may be concluded that the marked mitotic proliferation induced so readily in the thyroid of guinea pigs by potassium iodide is prevented by anterior pituitary gland when both are fed to the same animal. These two substances administered separately are antithetical in their action on the thyroid; anterior pituitary gland depressing proliferative and probably also functional activity as indicated by a decided drop in the mitotic count below the normal level and by the appearance of small acini filled with a dense deeply staining colloid, while KI causes marked stimulation, in the initial stage of its action at least, with enormous increase in the number of mitoses and with the appearance of a softened, pale, vacuolar colloid containing phagocytic cells. The condition of the thyroid which follows feeding of both these substances to the same animal is due to their direct action on the gland

itself, and not an effect which they exert upon each other before absorption from the gastro-intestinal tract, because changes in the thyroid are the same whether both drugs are fed simultaneously or whether one is fed in the morning and the other in the evening precluding any chance of direct interaction between them.

The stimulating influence of iodine and depressing property of anterior pituitary substance can be balanced in such a manner that an approximately stationary or resting condition in the thyroid results. In some respects anterior pituitary substance produces changes in the thyroid gland analogous to those induced by thyroid itself or by thyroxin. Loeb has shown that thyroid as well as pituitary substance prevents the compensatory hypertrophy that follows extirpation of a major portion of the thyroid gland, leaving open the question as to what constituent of the preparation of anterior pituitary (Armour & Co.) is responsible for this effect.

From these experiments it is impossible to determine the exact manner in which pituitary substance exerts its influence upon the thyroid gland. All that one can say is that anterior pituitary hormone in some way depresses the activity of the thyroid and holds in abeyance its capacity for responding to the stimulating effect of potassium iodide.

Summary

Oral administration of anterior pituitary tablets (Armour & Co.) causes a depression in the activity of the thyroid with a marked lowering of the number of mitoses in the entire gland and with medium-sized, or somewhat smaller, acini distended with hard colloid compressing the lining epithelium into thin strands of cells.

During the first stage of its action, potassium iodide, on the other hand, produces marked stimulation with enormous mitotic activity and a slightly softened colloid occasionally containing large numbers of phagocytic cells.

The early proliferative change induced in the thyroid gland by potassium iodide is prevented by anterior pituitary when both these substances are fed to the same animal.

Note: The author is indebted to Professor Loeb for suggesting this problem and for advice and assistance during its preparation.

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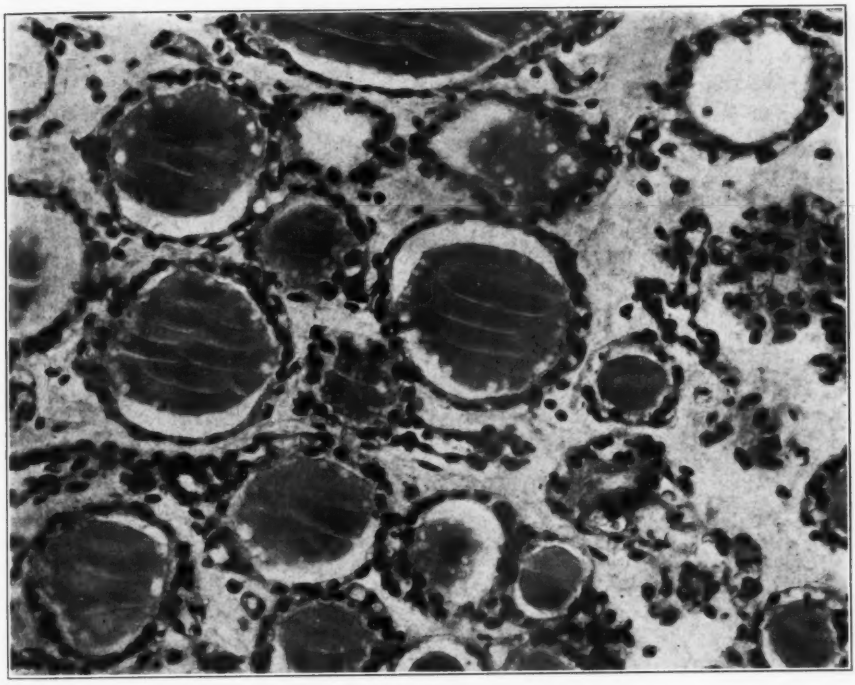
Loeb, Leo, and Kaplan, E. E. *J. Med. Res.*, 1924, xliv, 557.
2. Gray, S. H., and Loeb, Leo. *Am. J. Path.*, 1928, iv, 257.
3. Rabinovitch, Jacob. *Proc. Soc. Exper. Biol. & Med.*, 1928, xxv, 812; *Am. J. Path.*, 1928, iv, 601.

DESCRIPTION OF PLATES

PLATE 35

FIG. 1. Normal thyroid. × 280

FIG. 2. Fifteen days feeding with KI. Several mitotic figures are present near the center of the picture. × 280



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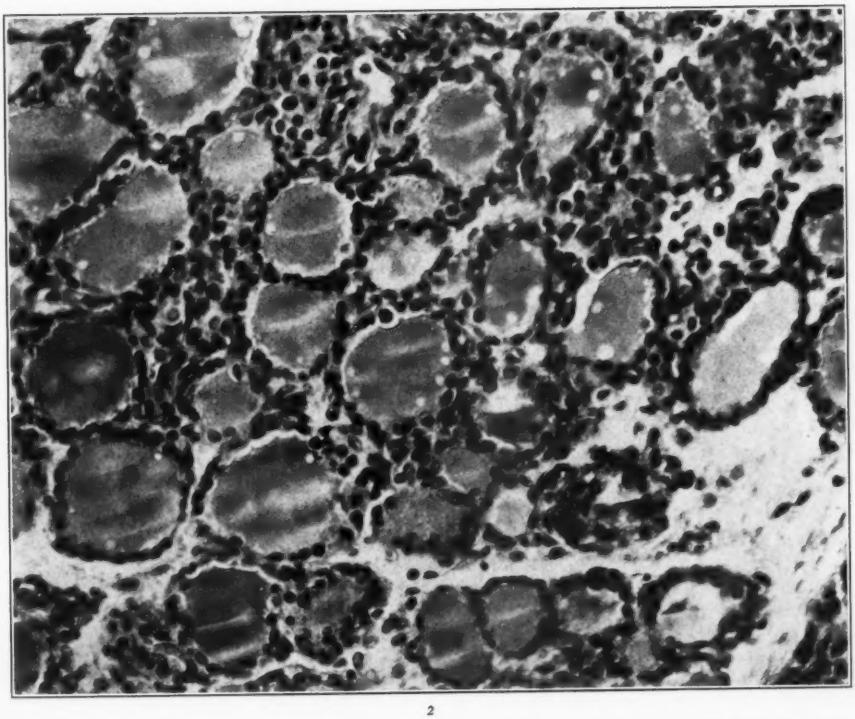

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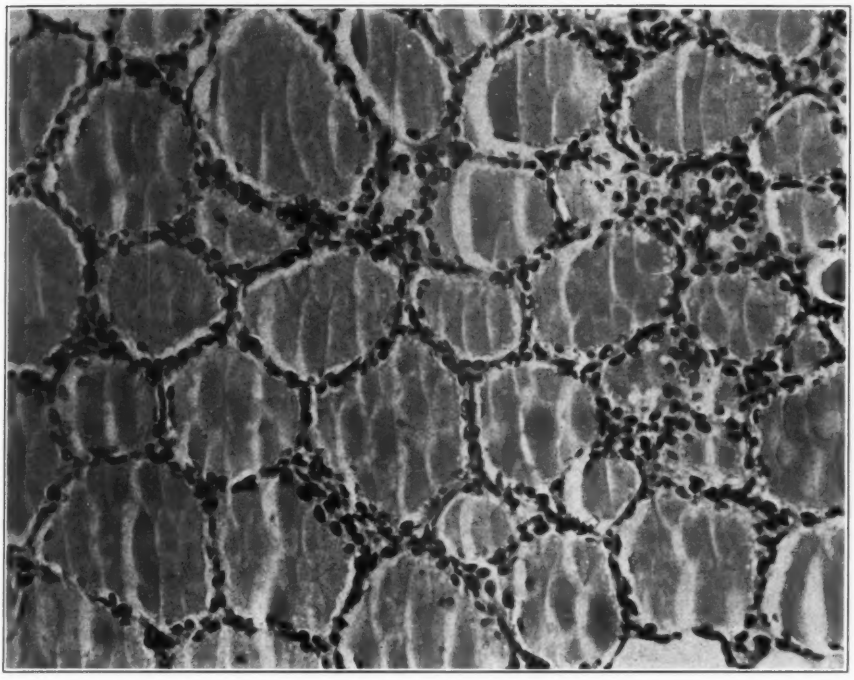
McCordock Thyroid Gland

PLATE 36

FIG. 3. Thyroid from animal fed anterior lobe of pituitary gland for fifteen days. × 280

FIG. 4. Combined feeding with KI and pituitary, fifteen days. × 280



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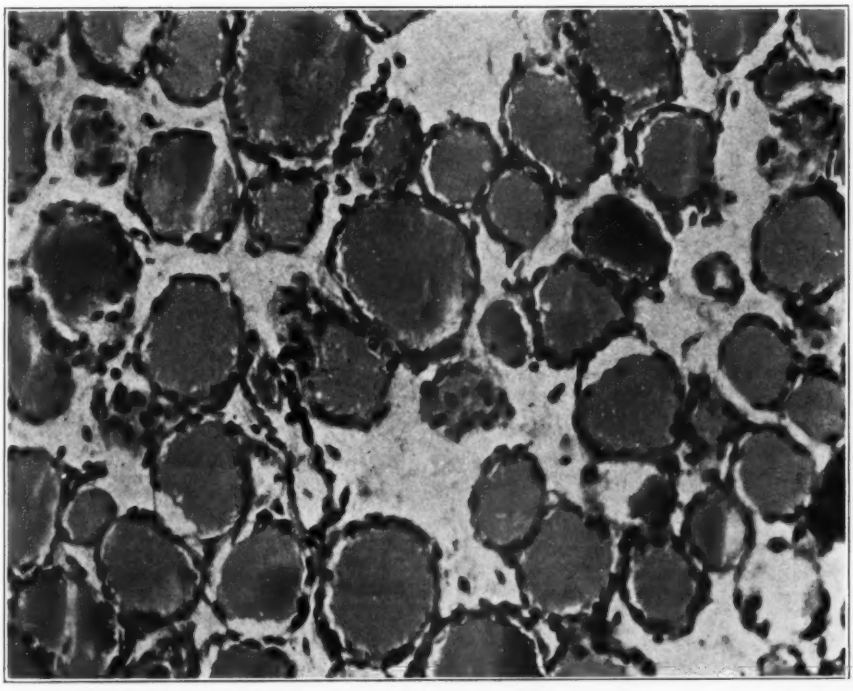

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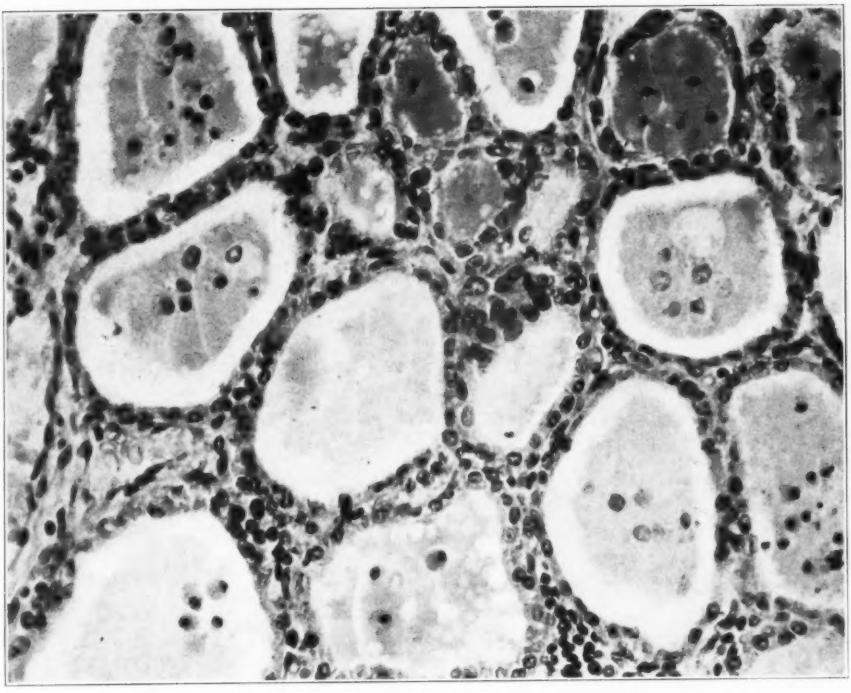
McCordock Thyroid Gland

PLATE 37

FIG. 5. KI feeding for twenty days. Compare with Fig. 2. Note large acini with soft colloid containing phagocytic cells and absence of mitoses and hypertrophic epithelium. × 300

FIG. 6. From same animal as Fig. 5. Note large acini. One in lower left-hand corner shows a constriction at the middle indicating the manner in which these large acini are formed by coalescence of two smaller ones. × 300



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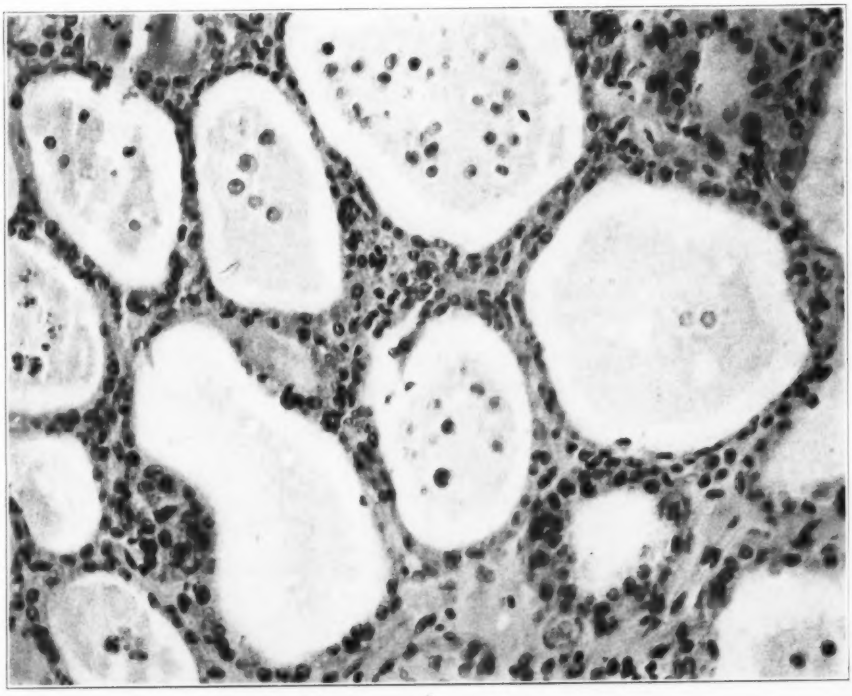

6

McCordock Thyroid Gland

FENESTRATIONS OF THE SEMILUNAR VALVES*

ARTHUR N. FOXE, M.D.

(From the Bellevue Hospital, New York, N. Y.)

The following is largely a statistical study of fenestrations of the semilunar valves of the heart in 300 successively observed hearts. With but few exceptions, the specimens were examined at the autopsy table.

The literature of this subject is scant and to my knowledge there are no numerical, statistical studies. From a review of textbooks of cardiology and anatomy, it is easily apparent that there is much confusion as to the nature and frequency of these valve defects. It seems to be generally accepted that they are of no clinical significance.

FREQUENCY

(*a*) There were one or more fenestrations in 82 per cent of all cases.

In	53	cases	no	cusps	were involved
"	71	"	1	cusp	was "
"	65	"	2	cusps	were "
"	39	"	3	"	" "
"	32	"	4	"	" "
"	30	"	5	"	" "
"	10	"	6	"	" "

(*b*) The aortic and pulmonic valves were involved with a negligible difference in frequency.

Aortic	in	188	cases
Pulmonic	in	187	cases

(*c*) In an estimation of the total number of fenestrations the frequency of the pulmonic involvement exceeded the aortic (pulmonic 1045, aortic 789); whereas in an estimation of the total number of cusps involved the reverse was true (pulmonic 314, aortic 325).

* Read before the New York Pathological Society, November 18, 1928.
Received for publication November 7, 1928.

Age

The age groups, average number of fenestrations per case and age frequency of patent foramen ovale are given in the following table:

Age Group	Number of Cases	Number of Fenestrations per Case	Per cent Patent Foramen Ovale
Fetus	3	1.3	66
2 years and less	20	.7	60
2–10 years	19	4.1	37
2nd decade	6	3.3	0
3rd "	25	5.9	12
4th "	51	8.0	22
5th "	82	6.3	13
6th "	62	7.0	21
7th "	29	7.8	10
8th "	3	2.5	33

It is evident that the frequency of fenestrations increases with age from the fetus to the fourth decade. After this there is a slight decrease, possibly due to the increasing frequency of sclerotic changes which obliterate small defects.

Of the cases under 10 years of age (42 cases), 40 per cent had no fenestrations. Of those over 10 years of age (258 cases), 14 per cent had no fenestrations.

There is no evidence to prove that fenestrations are developmental defects. It is even questionable whether there is any cause in an inherent endocardial or connective tissue weakness. The fenestrations form a pathology fundamentally mechanical in origin. Those few present at birth may be acquired from the mechanical effects of the intra-uterine fetal circulation. On the other hand, the frequency of patent foramen ovale, a distinct developmental defect, decreases with age and especially so after the first few years of life. This decrease may be due to later closure or to association with other developmental defects that lead to early death. In statistical studies of the frequency of patent foramen ovale it is essential that age groups be given. In this series of cases the frequency of this defect was 22 per cent.

It is interesting to note that in the three cases that showed marked developmental defects (bicuspid pulmonic valve, quadricuspid pulmonic valve, interventricular defects), there were no fenestrations; however, the latter two of the three cases had a patent foramen ovale.

Sex

In the female the valve leaflets are less frequently involved. In males, aortic, pulmonic, or both valves were uninvolved in 55 per cent; in females, in 64 per cent.

Etiology and Classification

Mechanical: (*a*) Circulatory (persistent strain of current).
(*b*) Traumatic.
(*c*) Ulcerative (infection plus strain of current).

Apparently the most frequent cause is the persistent strain of the blood current against the valve leaflets. It is questionable whether the perforations of ulcerative endocarditis or trauma (one case of each in this series, the latter due to a bullet wound) should be included in a classification of fenestrations. An understanding that the defects are all fundamentally mechanical in origin would justify such a classification.

Nature and Variety of the Fenestrations

The most frequent site of the fenestrations is adjacent to the attachment of the free edge of the cusp to the aortic intima. The fenestrations are usually ovoid apertures, with the long axis parallel to the free edge of the valve, and are apparently defects [1] in the endocardium that bridges the spaces between the connective tissue strands of the valve stroma. The defects may be single or multiple and may form large gaps in the valve surface. Across these gaps the remnant strands of fibrous tissue cross and intersect each other to form a veritable network which at times has a peculiar anatomic beauty.

The fenestrations occur less frequently along the free edge of the valve, and then as a rule well away from the corpora arantii. Due to adhesions of the free edges of two cusps the fenestration may permit no communication with the heart cavity, but form, instead, a passage between two sinuses of Valsalva.

Very large numbers of fenestrations are rare. In one case, a man aged 25, the aortic valve had 36 and the pulmonic valve 26 fenestrations. All of the leaflets were involved. Rarely the fenestrations are so fine as to defy count with the naked eye. This occurs in valves that have become markedly sclerotic and thickened.

Clinical Significance

Clinical data were lacking in so many cases that it was impossible to correlate any of the findings with murmurs, blood pressure readings, etc. In two cases the fenestrations were so large and numerous as to bring to mind the possibility of an aortic insufficiency. In one of these, with a non-dilated aortic orifice the fenestrations occupied at least one-half the valve area.

Summary

1. In a series of 300 cases one or more fenestrations occurred with a frequency of 82 per cent.

2. The frequency of pulmonic and aortic involvement was approximately equal.

3. Fenestrations, which are acquired defects, increase in frequency with age.

4. Patent foramen ovale, which is a developmental defect, decreases in frequency with age. The total percentage for the frequency of the occurrence of this defect was 22.

5. Classification, nature and variety of the fenestrations are briefly discussed.

6. It is possible that fenestrations may occasionally cause an aortic insufficiency.

My thanks are due Dr. Charles Norris, Chief Medical Examiner, and Dr. Douglas Symmers, Director of Laboratories at the Bellevue Hospital, for permission to study and report these cases.

Note: Since the submission of this paper for publication I have observed a large perforation of one of the aortic leaflets, apparently due to an atherosclerotic ulcerative process. Interesting also are the experimental perforations of the semilunar valves in animals. These were followed by evidences of aortic insufficiency (Herrmann, G. R. *Am. Heart J.*, 1926, i, 671).

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SO-CALLED INFECTIOUS SARCOMA OF THE DOG IN AN UNUSUAL ANATOMIC SITUATION *

WILLIAM H. FELDMAN, D. V. M., M. SC.

(From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.)

Few animal tumors have been the subject of more investigational study than the so-called infectious sarcoma of dogs. This growth is also known to veterinarians as "venereal granuloma" and as "venereal lymphosarcoma." The disease is not infrequently seen in kennels which house large numbers of dogs, and it occasionally appears in individual dogs not associated with kennel life. Practically all who have studied the condition agree that the primary focus of the disease is either the penis of the male or the vagina of the female, and that the disease is transferred to unaffected, susceptible animals by affected animals during the act of coitus.

Although the literature of the subject is known to many, it would seem apropos, in view of the unique site of the tumors in the case which constitutes the basis of this paper, to give a brief summary of the more important published reports concerning this interesting tumor.

Nowinsky [1] is given credit for the first published observations on this tumor. He reported a transplantable neoplasm which was removed from the vagina of a bitch and which recurred after a period of three months. He diagnosed the tumor myxosarcoma, and it was successfully transplanted subcutaneously into three puppies, aged eight days, fourteen days, and two months respectively. Metastasis was not observed.

Wehr [2] reported successful transplantation of the tumor which he considered a carcinoma. Geissler [3] also considered the growth to be a carcinoma, although Hansemann discussing Geissler's paper was of the opinion that the tumor was not a carcinoma.

Duplay and Cazin [4] were the first to suggest the possible inflammatory nature of the growth, although in one of the animals which bore successful transplantation of the tumor, metastasis to one testicle was noted. Bashford, Murray and Cramer,[5] after a careful study of

* Received for publication January 18, 1929.

the disease denied that it was neoplastic and, although they admitted that there was histologic resemblance between this growth and round-cell sarcoma, they concluded that "these tumors of the vagina and penis of the dog are connective tissue reactions to a living virus which has not yet been demonstrated."

In a series of papers dealing with this tumor, Smith and Washbourn [6, 7, 8] described an instance in which twelve bitches were served by a dog affected with a tumor on the penis. Vaginal tumors developed in eleven of the bitches. Three of the bitches which possessed transplanted vaginal growths were served by a second male on whose penis a tumor developed subsequently. The second male in turn served two unaffected bitches and a vaginal tumor developed in one. These authors also recorded a case in which a healthy dog was permitted to serve a bitch with a vaginal tumor and, in spite of the fact that the penis was frequently washed after coitus, a number of small growths developed on the part. They noted that transplanted growths were visible within one week after inoculation as small "glistening elevations about the size of millet seeds, mostly transparent but sometimes blood-stained. They looked like vesicles, but when they were pricked they proved to be solid." In three instances, Smith and Washbourn found the inguinal lymph nodes invaded with metastatic tumor cells, and in one dog metastasis was present in the liver and the spleen. They considered that the highly bred and more sensitive breeds, and dogs in poor general condition, offered more favorable environmental conditions for the growth of the tumor than were offered by the mongrel type or those that are well nourished. They noted spontaneous recovery with immunity to subsequent exposures in several instances.

From the results obtained in a series of inoculation experiments and from a general study of the disease, Smith and Washbourn noted that death may occur from "cachexia or from kidney disease, the result of obstruction of the ureters or from septic poisoning owing to sloughing of the growth." They concluded: (1) the tumors are infective round cell sarcoma; (2) they can be transplanted from the genitals, where they naturally occur, to the subcutaneous tissues of dogs; (3) they can be transplanted from the subcutaneous transplants in like manner through a series of dogs; (4) their spontaneous disappearance may occur with or without ulceration; (5) death may occur from metastatic deposits of the tumor in the viscera; and (6)

if the tumor disappears, the animal is then immune to subsequent inoculation.

Sticker,[9, 10, 11] who considered the growth to be a round cell sarcoma, made several important observations concerning its physiologic and pathologic aspects. He noted that successful transplants were possible in the spleen, bone and peritoneum, as well as in the skin. He was able also to secure successful transplants in two of three foxes which were used, but he was unsuccessful with cats, rabbits, guinea pigs and mice. In 16 per cent of all dogs with transplanted tumors in Sticker's series, the growths disappeared spontaneously, and these animals were immune to reinoculation. Sticker experimented with the viability of the tumor cells and found them quite resistant to extremes of temperature. They remained viable after twenty-four hours of exposure to a temperature of $-14°$ C, and after two hours of exposure to 50° C. On the other hand, attempts failed to produce growths from cells which were kept at $-11°$ C for twenty-five days and from cells which had been subjected to crushing. Neither did growths result from filtered or centrifuged emulsions of cells.

White's [12] observations concerning the contagiousness and malignancy of the tumor are of interest. Two pure-bred bull bitches were served by an apparently healthy male, and vaginal tumors developed in both shortly after whelping. In one of the dogs, a mammary growth also appeared. Both bitches were operated on and after removal of the tumors they recovered completely. They remained well, were bred twice afterward, and each gave birth to normal litters. The male used to breed the first two bitches also served four other females, in all of which the growths developed and they died in consequence of them. One of the first two bitches was served by three other males, and all died of the disease.

White believed that these growths have several features in common with infectious diseases, but he concluded that the growths which occur following inoculation have their origin from the transplanted cells and not from a separate infective agent. White also pointed out certain differences between the tumor and the usual forms of sarcoma. He mentioned the marked contagiousness of the venereal growths and the relatively mild malignancy of the majority of these tumors in that they show little tendency to infiltrate the surrounding tissues and do not often produce metastasis.

Beebe and Ewing [13] undertook to show by experiments with transplantation the exact manner of origin of the tumors which resulted. They transplanted small grafts of tumorous tissue subcutaneously and made histologic studies of excised portions at intervals of one to twenty-one days. This was done in an attempt to determine "whether the growth comes from the proliferation of the transplanted cells or by stimulation of the surrounding tissue cells as maintained by Bashford, Murray and Cramer." Beebe and Ewing found that in transplants all the tumor cells do not die; some at the periphery remain alive. These undergo mitosis and "leave no doubt that the transplanted cells give rise to the tumor." Concerning the nature of the disease, these writers were "forced to conclude that the infectious lymphosarcoma of dogs is a true malignant neoplasm." They admitted, however, their inability to determine the exact tissue cells involved in the histogenesis of the tumor. At the time of Beebe and Ewing's report, the tumor with which they worked was in its fifth generation, and thirty-five tumors had developed in the twenty-nine dogs used. Their paper includes a rather complete description of the clinical aspects of the disease, and they stated that in both sexes isolated tumors may occur anywhere over the body as well as in the spleen, kidney, liver and lung.

Wade [14] also conducted a rather exhaustive investigation of the disease during the course of which he successfully inoculated two fox cubs. The animals died, much emaciated, after the tumors had attained the size of about 2 by 1 cm.

Rabbits, rats and guinea pigs resisted successful transplantation of the tumor, and six dogs that had recovered spontaneously from previous successful inoculations apparently were immune to attempts at reinoculation. Wade observed interstitial nephritis in many successfully inoculated dogs, and he thought this lesion represented the effect of a soluble toxin from the tumor cells and constituted the strongest evidence advanced up to that time in favor of the disease being an infective granuloma and not a true neoplasm. It was Wade's opinion that the growths which occurred as a result of inoculations of tissue had their origin in part from the vigorously dividing peripheral cells of the tissue which was introduced, and in part from the surrounding connective tissue fibroblasts which become altered in the process of repair and assume an immature sarcomatous nature. He therefore considered the growth to be an

infective sarcoma, a tumor belonging "to the borderline between the infective granulomas and the true neoplasms." Those tumors which arise after natural infection Wade believed to consist of cells derived entirely from the tissue of the host.

In another report by Beebe,[15] the correctness of considering these growths to be of an infectious nature was questioned. Beebe felt that evidence in support of the action of microörganisms in their genesis was lacking and brought out the fact that successful transfers of the tumor had been accomplished only by means of a living tumor cell. Beebe again expressed his belief in the true neoplastic nature of the process.

Crile and Beebe[16] subscribed to the view that these growths are neoplastic as follows: "From the total available evidence we are convinced that the process is a true tumor." These workers made some extremely significant observations concerning the curative value of blood from animals that had recovered spontaneously. In brief, they effected direct transfusion of whole blood from an animal that had recovered spontaneously and therefore was immune to an animal with an actively growing tumor. Of ten tumorous dogs that were the recipients of transfused blood, seven were cured, and in two the transfusion apparently produced marked inhibition or regression of the growth. Crile and Beebe did not think the protective or curative properties that were exhibited were analogous to bacterial immunity; nevertheless, they thought that there was present a specific factor capable of rendering an affected animal passively immune.

Two other papers that have to do with the use of certain biologic products in the treatment of these tumors were written by Bergell and Sticker,[17] and by Beebe and Tracy.[18] Bergell and Sticker were able to cause complete disappearance of the tumor, in cases in which spontaneous recovery was unlikely, by injection of a specific hepatic ferment. In one case, with metastasis to lymph nodes, injection of the hepatic ferment caused regression which extended to the involved node. Following the injection, the tumors became cystic with marked phagocytic action by macrophages, and, in place of the numerous mitotic figures which characterize the tumor in its progressive stage, the injected tumor was quite devoid of mitotic figures. Beebe and Tracy demonstrated the destructive influence of certain bacterial toxins on the tumor. Tumors at a distance from the in-

jected growth regressed simultaneously with those injected. In one instance, an animal with four transplanted tumors received four injections at some distance from the growths, and six weeks after the first injection the tumors had completely disappeared. The authors were not convinced that the effect produced was peculiar to the bacterial toxin used, since they closed by saying, "It seems possible . . . that an equal bulk of any toxic organism would exert a similar destructive action."

Novak and Craig [19] recently reported one case, and after reviewing the literature they concluded that the weight of evidence seemed to substantiate the contention of Beebe and Ewing that these growths are true tumors and not inflammatory granulomas.

Attesting to the universal distribution of the disease is the statement of Seligmann [20] that the disease was endemic in New Guinea before the advent of the white man and hence was not dependent for its appearance on imported dogs.

Since the disease as observed by the investigators mentioned involved the genitals, the finding of tumors histologically similar in every detail to the so-called infectious lymphosarcoma, but in regions entirely apart from the genitals, is considered of sufficient importance to justify the report which follows.

Report of Case

The animal was a mongrel female terrier pup of unknown parentage. In the latter part of June 1928 when she was approximately four months of age the right eye appeared to bulge outward. On examination the eye was found to be opaque and of a cream color, and it seemed to be crowded forward by pressure from behind. Soon after this observation a small nodular tumor was noted under the skin of the frontal region, slightly to the left of the median line (Fig. 1). The opaqueness of the eye gradually changed to a bright red and August 22 a lobulated tumorous mass occupied the bulk of the orbital space, and the eyeball could not be seen. The frontal tumor grew rapidly and August 22 it measured 4 cm. in diameter and was raised above the surrounding tissue 3.5 cm. The frontal mass apparently occupied a subcutaneous position without involving the underlying bone. Other tumors were not observed elsewhere over the body.

September 11, 1928, the tumor of the eye, which had been growing vigorously, had become badly infected and for humane reasons it was decided to attempt surgical removal of both tumors (Fig. 2). This was done under general anesthesia. The eye mass was broadly attached to the posterior portion of the orbit, and the eyeball was completely embedded in the more anterior part of the tumor. The growth was very vascular and the resultant hemorrhage was profuse. The tumor in the frontal region was found to lie just under the skin and to be firmly attached to the underlying bone of the skull; this made complete removal difficult.

Grossly, the tumors were of firm texture and of a grayish white color. At the time of the removal of the tumor the mass in the eye seemed more vascular than the frontal tumor, whereas the latter was of firmer consistence.

From the beginning of the disease the general condition of the dog was excellent. She remained frolicsome and apparently had normal bodily growth. Notes on the animal's subsequent condition follow:

September 24. Both wounds are healing satisfactorily; the animal is in good spirits.

October 10. The frontal wound is nearly healed; the orbital wound is discharging a thin, purulent fluid.

October 30. The frontal wound is completely healed. The orbit continues to discharge a thin grayish fluid.

November 27. There is no apparent recurrence in the frontal region. A small amount of fluid is still draining from the eye socket. Recurrence of the growth that involved the eye cannot be demonstrated.

December 26. There is no recurrence of either tumor. The space from which the tumor of the eye was enucleated has healed completely, although a superficial watery discharge persists (Fig. 2).

Immediately after the removal of the tumors from the body and before their exact nature was determined, they, unfortunately, were placed in fixative fluids, and this precluded possible attempts at transplantation, the desirability of which was made evident by subsequent observations. It is regrettable that a portion was not reserved for experiments with transmission since the subsequent diagnosis revealed a type of growth similar in all its morphologic details to the so-called infectious lymphosarcoma (Figs. 3, 4 and 5).

Microscopic Examination

The tissue in the tumor of the eye consisted of rather large irregularly shaped cells inclined to be polyhedral in outline. They were closely packed together and the continuity of the cell masses was interrupted by strands of fibrous tissue of variable thickness which supported the numerous blood vessels of the growth. Aside from the fibrous strands just mentioned, there was no demonstrable stroma supporting the cells of the tumor (Fig. 6). There was much extravasation of blood in some of the fields and necrosis with some edema on the surface of the growth. Some of the larger vessels near the surface were thrombosed. The cytoplasm of the neoplastic cells was finely granular and was moderately acidophilic in staining quality. The nuclei, which represented about half of the cellular bulk, possessed a large number of coarse chromatin granules, and there was marked affinity of the whole structure for the basic stain.

Mitosis was present in many cells in practically every field, and several interesting phases of this phenomenon could be seen. A prominent, slightly eccentrically located nucleolus could be seen in the majority of cells (Figs. 6 and 7).

The cells were markedly invasive, and the tumor had every structural feature characteristic of a highly malignant tumor. Attempted encapsulation or other evidence of resistance on the part of the normal tissues was not apparent.

The elements present in the tumor of the frontal region, and their arrangement, were an exact replica of the tissue which constituted the tumor of the eye (Fig. 5). It was noted, however, that that portion of the tumor nearest the skin was covered with a dense layer of fibrous tissue of considerable thickness, which contained a large number of small vessels. Inflammatory leucocytes were absent from this tissue. Although for the most part the fibrous connective tissue appeared to be of an adult type, a few areas could be seen near the substance of the tumor where the fibroblasts were somewhat less mature. In these areas some large wandering cells were present between the loosely arranged strands of tissue.

From a study of the material from both tumors it was at once apparent that the growths were similar and that they resembled in every detail the tumor of dogs previously designated infectious lymphosarcoma or venereal lymphosarcoma.

Discussion

This case is of little interest because the situation of the tumor was unusual. In the literature reviewed only one reference was made to the occurrence of these tumors in regions apart from the genitals. Beebe and Ewing mentioned that isolated tumors may appear anywhere over the body, but they failed to describe such a case. In reply to a communication concerning this point, Ewing [21] wrote as follows: "I have the impression that infectious sarcoma of dogs has been observed in other regions than the genitals. In fact, I think I have seen one case. . . ." Considering the large number of cases studied by the various investigators, and considering that cases of extragenital involvement have not been described, such cases must be extremely rare.

Consideration of the various features of this case leaves many important points unsettled, and opens anew the question of primary

importance in the study of such tumors: What is the exact histogenesis of the type cell constituting these growths?

Lack of knowledge of the parentage of the subject of this report makes it impossible to assemble all the facts one should possess before attempting an hypothesis concerning histogenesis. In this connection, there are at least two points of importance. In view of the facility with which the cells of this particular tumor lend themselves to successful transplantation, the possibility that the tumors in this case are the result of maternal inoculation at the time of birth should be considered. In case the mother possessed a vaginal tumor of this variety, the mass would be subject to considerable trauma at the time of parturition, and it is conceivable that viable cells from the parent tumor could find their way into the natural recesses of the eye, where favorable conditions would permit their multiplication.

The growth of the frontal region might arise in a similar manner, although it is difficult to imagine natural inoculation taking place through the unbroken skin, even through the tender skin of a newborn puppy.

The other possibility of the origin of these growths is that one or both had spontaneous origin from the tissues of the involved parts. Proof is lacking to substantiate origin by inoculation from the vaginal passage, and likewise it is impossible to present acceptable evidence that the tumors originated from cells derived from the tissues of the respective regions. There can be no doubt but that the tissues of the dog are capable, under the proper influences, of producing this peculiar autonomous cell. Whereas the disease usually is the result of the direct transference of living cells from the tumor of one animal to the tissues of the other, the condition must occasionally have a spontaneous origin; otherwise it would not exist. In further support of their non-transplantable origin is the observation of Seligmann that this tumor affected native dogs in New Guinea before the arrival of imported dogs.

There does not appear to be anything peculiar to the tissues of the genitals that would limit the spontaneous origin of this tumor to these regions, and it would seem quite proper to assume that the elements necessary for its inception may and probably do exist elsewhere. Since the tumors so far reported have appeared beneath the mucosa or under the skin, it is possible that the progenitors of the type cell have their origin as part of a natural response to some ex-

traneous stimulus. As far as can be determined, such tumors have not been encountered as primary growths in the tissues of the interior of the body. These tissues are spared much of the injury to which the exterior of the body is subjected. Furthermore, the partticularly violent exertions associated with coitus in the dog render both sexes prone to injuries of the genitals.

The multiplicity of the tumors in the case reported resulted from one of two possibilities. Either the growths arose independently of one another or one was a metastatic expression of the other. Whereas, of these two possibilities, it is perhaps easier to accept the idea of metastasis in explanation of the growth, it is not clear which of the two tumors antedated the other. Functional embarrassment was first noted in the eye, although the subcutaneous mass became evident soon afterward. If one of the tumors was the result of metastasis, the extension occurred very early in the growth of the tumor. After they became established, the masses grew with equal facility, but the tumor of the eye was inhibited perhaps by the limiting confines of the orbital space.

All who have studied these tumors have attempted to classify the type cell, and, although a few of the early observers thought the tumor was a carcinoma made up of epithelial cells, the opinion of the majority is that the growth should be considered a sarcoma.

If it is a tumor derived from cells of the lymphocytic series, it possesses certain features which differentiate it from the entity usually designated as lymphosarcoma. The cells are perhaps larger and more uniform in size, and an intercellular substance has not been demonstrated. The cells lie in direct apposition, and the cytoplasm of many of them is inclined to be pale or even clear (Fig. 6). The latter feature may, however, be the result of some retrogressive change and may not be observable in cells which are growing vigorously. Comparing this tumor with certain examples of rapidly growing carcinoma, one cannot avoid the thought that, morphologically at least, they have some features in common. Proof of definite relationship is lacking, and from a morphologic standpoint the tumor probably will retain a position between the highly malignant carcinomas and the lymphosarcomas.

The tumor lacks the viciousness one ordinarily would ascribe to other growths which possess many of the same evidences of malignancy, and death seldom ensues as a direct consequence of the

tumor. I have had under observation a dog in the vaginal vault of which such a tumor has persisted for nearly two years; and, while the tumor is slowly growing, the general health of the animal remains good and metastasis cannot be demonstrated. The failure of the tumor seriously to influence the well-being of the animal was a notable feature in the subject of this report. The dog had a normal bodily growth and remained well nourished and playful throughout the period that the tumors were present.

The ease with which this tumor can be successfully transplanted to other dogs, and the manner in which the disease responds to surgical interference, certainly place it in a unique position among the other neoplastic diseases. Considering its unusual clinical behavior, it is not surprising that the cells of the tumor should be found to be somewhat different from other malignant neoplasms. The origin of the type cell is difficult to determine. Although, morphologically, it does have some resemblance to cells such as occasionally are seen in squamous cell carcinomas of extreme malignancy, I am inclined to believe that the cells of this tumor arise from certain undifferentiated lymphocytic forms and that the growth represents a type of lymphoblastoma which can be fittingly designated "transmissible lymphosarcoma." The word "transmissible" is justifiable because of the high percentage of takes secured with this tumor in experiments with transplantation and the striking manner in which it is transmitted to susceptible dogs by sexual contact. These tumors should not be referred to as infectious sarcomas or as infectious lymphosarcomas, since there is no reason to assume that a specific infectious agent is responsible for their causation or that they are transmissible for the same reason. They apparently are transmissible because of the ability of the transplanted or inoculated cells to grow in the tissues of the recipient and not because of the transference of a separate infectious substance in the strict bacteriologic sense.

Summary

The more important literature pertaining to the transmissible lymphosarcoma of dogs is reviewed. A case is described in which two tumors, which morphologically were indistinguishable from transmissible sarcoma of the genitals, appeared respectively in the orbital space and under the skin of the frontal region of a young dog. The genitals were not affected. Although transplantation was not at-

tempted, there seems sufficient histologic evidence to substantiate a diagnosis of transmissible lymphosarcoma. The word "transmissible" is preferable to the older term "infectious," since there is not evidence bacteriologically or otherwise that these growths are dependent for their origin on an infective factor in the accepted sense of the word. It could not be determined whether each growth had a spontaneous origin or whether one represented a metastasis from the other. The possibility of the tumors being the result of transplantations from a vaginal tumor of the mother at the time of birth was considered, but this was not subject to proof since the parentage of the puppy could not be traced.

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DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Appearance of the tumors at the time of operative removal, approximately three months after they were first seen.

FIG. 2. The animal 104 days after removal of the tumors. The wounds had healed completely and recurrence was not apparent.

FIG. 3. Section from a typical transmissible or venereal lymphosarcoma from the vagina of a dog. × 660. A similarity may be noted in Figs. 3, 4 and 5.

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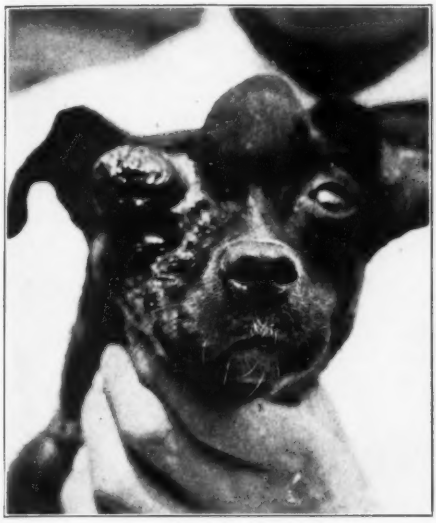

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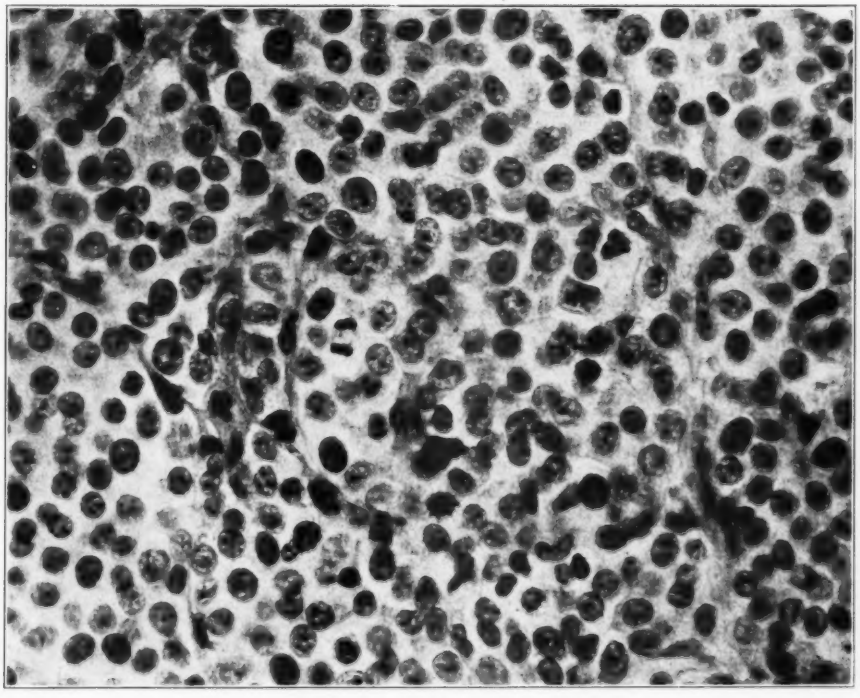

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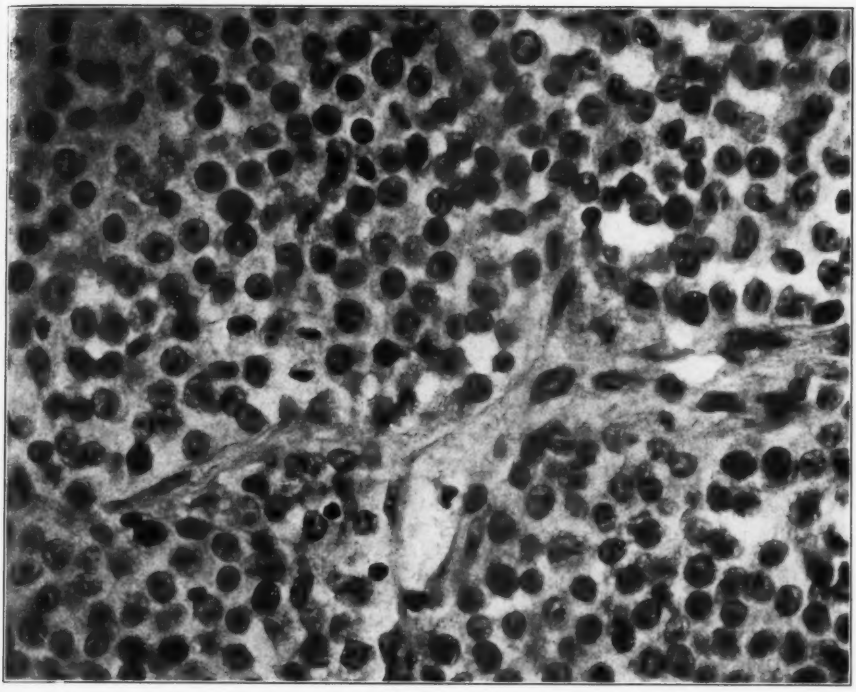
PLATE 39

FIG. 4. Section of the tumor of the eye in the case reported. The close resemblance to the genital tumor in Fig. 3 is apparent. × 660.

FIG. 5. Section of the tumor from the frontal region in the case reported. The structure is similar in every detail to that shown in Figs. 3 and 4. × 660.



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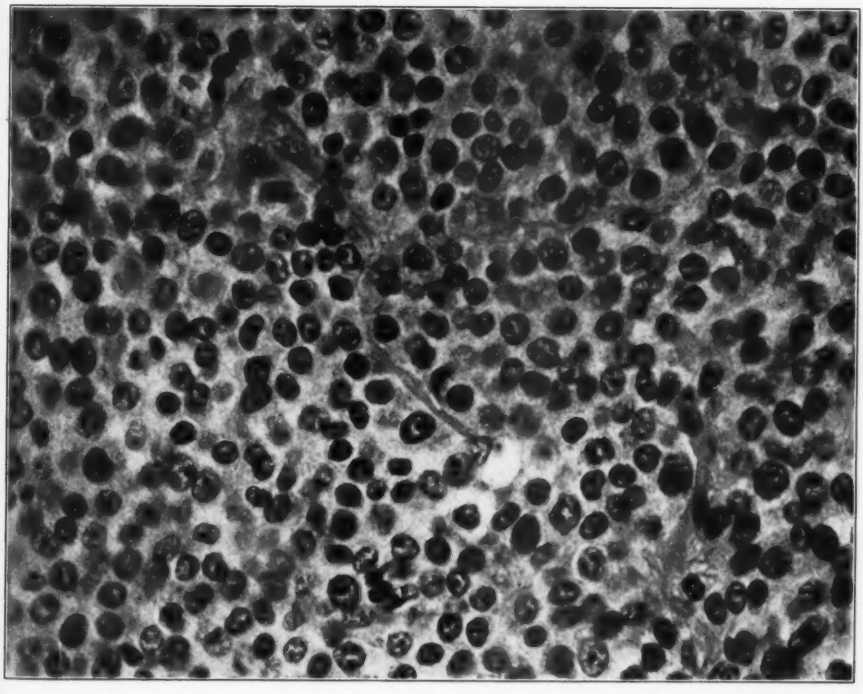

5

Feldman Infectious Sarcoma of Dog

PLATE 40

FIG. 6. Section of the tumor of the eye, showing the polygonal outline of the cells and their close apposition. The relative proportion of the nuclear substance to the cytoplasm is shown. One cell is in mitosis. × 2000.

FIG. 7. Section of the tumor of the eye showing the hyperchromatic nuclei and prominent nucleoli. One cell is in mitosis. × 1350.

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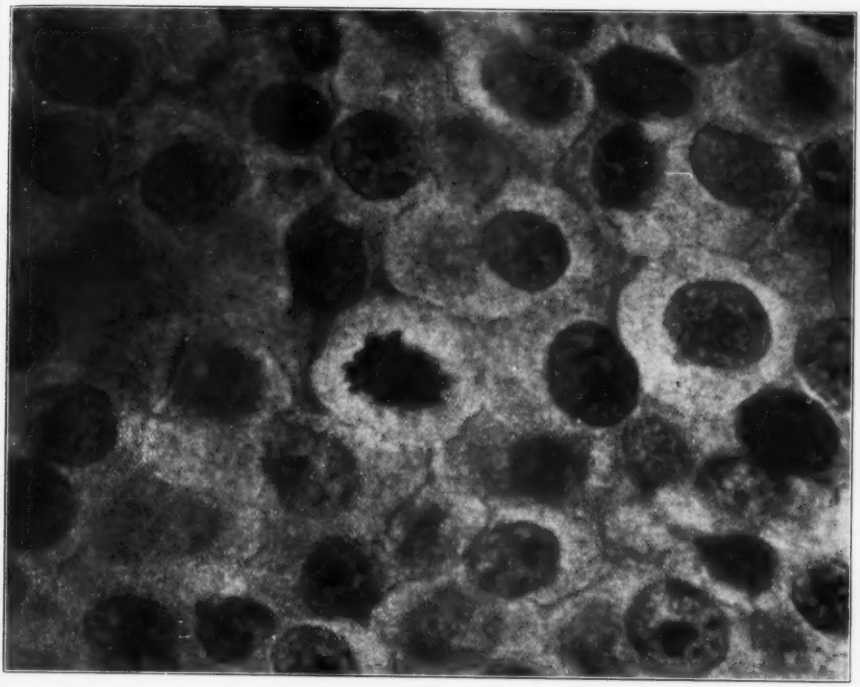

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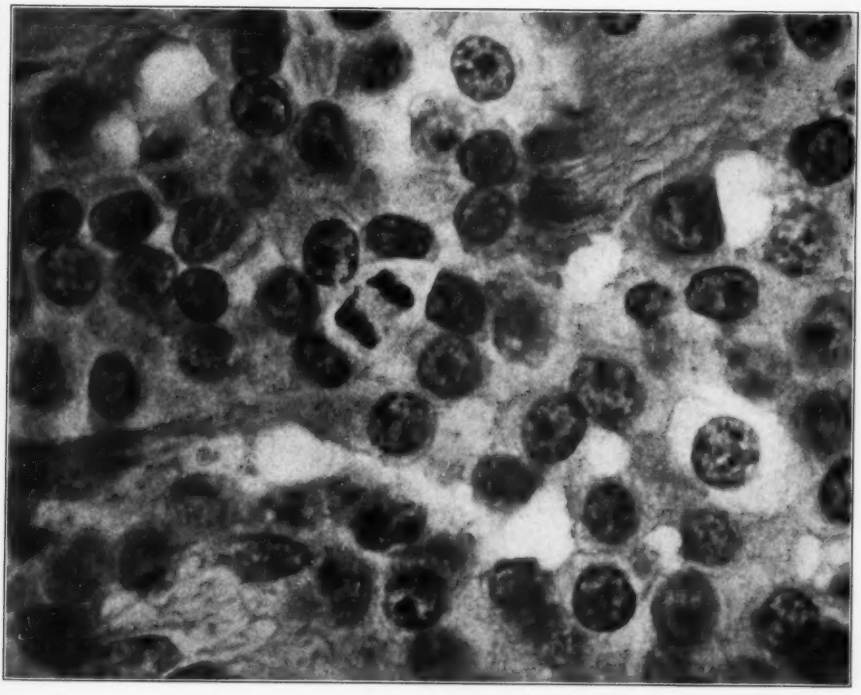

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Feldman Infectious Sarcoma of Dog

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